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## SCIENCE AND NUTRITION





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# SCIENCE AND NUTRITION

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WITH A PREFACE BY

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## PREFACE

AT no time has Brillat-Savarin's aphorism " *La destinée des nations dépend de la manière dont elles se nourrissent* " been more apposite than it is to-day. The survival of democracy or its annihilation during the next few years may easily be determined by the measure of attention given in the various countries to what have come to be called the problems of human nutrition.

What are these problems? Thirty years or more of experimental research have revealed that health is quite as seriously menaced by ill-balanced or defective diets as by germs, viruses and other infective agents. Perhaps in the former case the danger is even greater, because most infections betray their presence by a recognizable group of symptoms calling for treatment, whereas the onset of disabilities due to improper diet is often so insidious that irreparable harm may be caused before the alarm is raised.

The story of the remarkable developments in the field of experimental nutrition during these thirty years or so is simply and accurately told by Mr. Bacharach in the following pages. He speaks with the authority of one who has not only been in close touch with all the important research work of recent years, but who has himself made many valuable experimental studies. His easy and lucid style will at once appeal to the intelligent "man-(and woman-)in-the-street," for whom I am certain this book has been written. I hope they will read it in their thousands. I hope they will learn from its pages that the great majority of the "problems of nutrition," about which one hears so much nowadays, have been solved by the scientist, and that they are now translated into problems of economics or political science.

Let me give one example. In every part of the world where the very poor have been forced to live on maize as their staple food, there is found a repulsive and often fatal disease known as pellagra. For many years it has been attributed to faulty diet, but the experts could not agree whether it was caused, by a poisonous substance in the

deteriorated grain, by the poor quality of the maize proteins, by a vitamin deficiency or by one or other of many hypothetical defects. Within the past year a brilliant series of researches in which *rattus norvegicus*, to whom—I refuse to say “to which”—the author has so charmingly and appreciatively dedicated his book, played his modest but essential part, has proved that the disease arises from a deficiency of a substance called nicotinic acid, related curiously enough to the highly poisonous ingredient of tobacco. In this compound, which can very easily be produced by synthesis in the chemical laboratory, we now have a certain cure for pellagra.

But important as this discovery is, the main problem does not end there. The foodstuffs which contain most nicotinic acid, and which therefore give protection against the disease, are dairy produce and fresh meat. When these form a reasonable proportion of the diet there is no need to think in terms of nicotinic acid. Not so many years ago pellagra was rife in the Republic of Georgia. By making it possible for the inhabitants to obtain ample supplies of cheese and eggs, the Soviet Government has practically wiped it out. Conversely, we find that the incidence of pellagra in the “corn-belt” of the United States still sharply reflects the rise and fall of poverty and unemployment. It is not a question of nicotinic acid; it is simply one of *£ s. d.*

The only real problem of nutrition in England to-day is the task of making available to the poorest person the “protective foods,” such as milk, butter, fresh salads, and fruit, which are essential for health.

This book should be read not only by the intelligent “man-in-the-street,” whom for no very clear reason one assumes to be a layman, but also by the medical profession, until quite recently lamentably behindhand in recognizing the significance of experimental dietary studies, and perhaps even by the politicians who are at the moment very timorously testing the strength of “nutrition” as a party plank.

J. C. DRUMMOND

June 1st, 1938

## INTRODUCTION

NUTRITIONAL Science, especially on its experimental side, with which this book is mainly concerned, is a borderland subject, where chemistry and physiology meet, often along with physical chemistry, and even physics—to say nothing of contacts with zoology and botany (including bacteriology) and the potent weapon of statistics. It is—let us face the fact—a branch of biology, for it is concerned with what happens when certain living organisms kill, eat and inwardly digest (or try to) certain other living organisms.

This being so, I fully realize that the predominantly chemical treatment here given to the subject lays me open to criticism from many quarters—more especially from my physiologist friends. To them, therefore, in particular, I put forward both an excuse and (I hope) a justification. The excuse is that I happen, by training and profession, to be a chemist, and that merely prefixing to that designation the syllable “ bio ” cannot be expected to alter fundamentally the outlook engendered by thirty years’ training and practice. The justification is one of both expediency and principle. The interested and educated layman of any class, for whom this book is written, may quite possibly have once learnt some chemistry—at school, at night class, at college, at extension lecture, at university—and may even remember something of what he has learnt. The chances, alas, that he will ever have been instructed even in the earliest rudiments of animal physiology are still to-day so small as to be negligible. I think, therefore, that for him the chemical approach is likely to be less forbidding than the physiological. I am by no means the first to recognize this, nor have physiologists themselves been blind to it. In the many admirable publications of my friend, Dr. T. R. Parsons (notably in *The Materials of Life* and in the evergreen and ever-tended *Fundamentals of Biochemistry*), though the outlook is essentially physiological—that is, biological—the treatment is very largely chemical.

I think the reason of principle must have operated in

him also. Walter Pater said that all art aspires towards the nature of music; is it not also true to say that all biology aspires towards interpretation in terms of atomic physics? It will probably be conceded that chemical interpretations are a little nearer to the physical asymptote than are physiological. If so, the urgent need to guide the layman away from the mists of obscurantism—naturalism, vitalism and what not—in his application of scientific knowledge to ordinary human and social problems must surely justify the presentation to him of biological facts in terms as far removed as possible from the dangers of mystification. I honestly believe that there is a better chance of doing this on the chemical than on the physiological plane, fully realizing that this belief may have been largely conditioned by my own chemical training. That, at any rate, is my case.

It is customary for the author of a book in any introductory note to make acknowledgments of various kinds. If my thanks seem exiguous to the point of parsimony, let it be attributed to the fact that I know neither where to begin with them nor where to leave off. I am most grateful to Dr. Maurice Newfield and Dr. R. A. McCance, who read and helpfully criticized certain parts of the manuscript. Professor Drummond's kind Preface is perhaps the least of his services—to me and to the reader: he has read the whole of the manuscript and saved me from error or obscurity on many occasions. He and those others who have helped me are, however, in no way responsible for my statement of any facts or for my arguments from the facts. They must be credited with much of any virtue in this book; they must be debited with none of its faults. To Professor H. Levy, in a sense the godfather of this book, to my wife and to the publishers I feel that no adequate acknowledgment can be made of their patience, aware as I am that over the book I have been an unconscionable time writing.

Finally, to the editors of various journals that have thought fit to publish reviews, articles and notes of mine, I apologize for any unintentional infringement of their copyrights, appreciating that, in re-stating here

facts and arguments already stated there, I may well have inadvertently repeated phraseology to an extent not to be covered by "reasonable quotation." My acknowledgments on this score are particularly due to the editors of the following journals: *The Analyst*, *Chemistry and Industry*, *Food*, *Food Manufacture*, *The Industrial Chemist*, *The Manufacturing Chemist*, *Nature* and *The New Statesman*. Part of the last chapter appeared in substantially the same form in *The Highway* for April, 1938. I am much indebted to the Editor of *The Lancet* for permission to reprint the Annotation that appears on pp. 137 and 138.

*July, 1938*



## INTRODUCTION TO SECOND EDITION

THAT a new edition of this book is needed must be laid to the charge of the war. My critics may consider it one of that calamity's minor evils: my friends—and I hope my new readers—an infinitesimal consolation. The dearth of books and the shortage of reading matter themselves have caused almost every published work to go out of print, so that many publishers have found themselves in the delightfully embarrassing position of having to decide whether to issue new books or simply to re-issue old ones, knowing that they have not the paper for both. This book, therefore, has taken its place along with hundreds of much more important and profound works in the queue of temporarily silenced voices waiting to become vocal again by becoming visible as ink and paper. Furthermore, the last five years have shown a very marked change in the attitude of the public to the problems of nutrition. An enlightened Ministry of Food of which the effectiveness was no doubt in large measure due to its youth and its freedom from the trammels of precedent; to a Minister who not only understood human nature, but also appreciated the value that science can have for the community; to a scientific adviser—the writer of the Preface to this book—whose grasp of practical possibilities proved as shrewd as his knowledge of nutritional science was wide and flexible, and whose practical and scientific services have, since this book was first published, been fittingly rewarded by Knighthood and the Fellowship of the Royal Society; last, but by no means least, to a Public Relations policy that has been as persistent and enthusiastic as it has, generally, been honest and intelligent: all these factors have helped to make tens of thousands of people realize for the first time that eating and drinking may be as important in their effects on our physical health as a few have always realized them to be on our spiritual well-being. Among those who are now willing to accept, if not instructions, at least instruction from the nutritional scientist, there will

surely be some, and there may indeed be many, who would like to probe a little into the reasons that underlie the advice they are given and will be more willing to accept it, if they understand its scientific basis. It is hoped that this book, which has been thoroughly revised and in parts rewritten for the new edition, may help them to do so.

The revision has been made much easier than it would have been, because a number of friends and colleagues took the trouble to call my attention to various "errors" in the first edition. In nearly every instance they were right and I was wrong—sometimes egregiously. In all such instances I have corrected the errors—or tried to—and have also thanked the correctors, privately. I should like to do so here, publicly.

*August, 1944*

### NOTE TO THIRD EDITION

Comments of critics and friends—often, I am glad to say, the same person—have led to a number of changes in this edition. They are not very extensive and will probably only be detected by those who look for them. All the same, I am very grateful to their originators. I have also included some account of folic acid, of which the formula has been published and the synthesis described since the second edition of this book went to press.

*June, 1947*

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## SECTION I—THE EXPERIMENTAL BASIS

### CHAPTER I

#### INTRODUCTORY

§ 1 THE object of this book is to describe, as simply as the size of the book and the nature of the subject permit, both the results obtained and the methods used in scientific investigations, especially those carried out under laboratory conditions, that have influenced our views of the relationship between human nutrition and health. It is customary to jeer gently at publications which try to show in precise terms how inadequate feeding, qualitative at least as much as quantitative, is exactly correlated with general and specific ill-health, on the grounds that this is simply gilding the lily and stating in a complicated way what all open-minded people know to be the simple truth. I think the jeerers are wrong—wrong in fact, for the correlation is by no means as widely accepted as they would like to believe; wrong in intellectual approach, for they fail to distinguish between vaguely accepted generalizations and the exact bases of those generalizations, which alone can turn vague acceptance into effective corollary action. So that they are thirdly wrong as well—wrong tactically, as social reformers, politicians and revolutionaries.

§ 2 In order to carry out our object it will be necessary to try to steer a very careful course between scientific inexactness on the one side and excessive detail on the other. I therefore apologize in advance to those who will be inclined, doubtless with frequent justification, to criticize me from the safe heights on either side of these narrow straits. To the scientist, and especially to my biochemical colleagues, this book will sometimes appear to be lacking those meticulous qualifications that are essential in accurate technical expositions; the layman, on the other hand, may occasionally find himself asked

to take for granted matters that are by no means self-evident.

It is, however, for the layman that this book is mainly intended. I must, therefore, direct his attention to the fact that there are many other books, of not too specialized a nature, from which he will be able to supplement such new information as I may be fortunate enough to give him, should I be successful in arousing in him the necessary interest. In any event, if I occasionally credit him with knowledge that he does not happen to possess, and omit some connecting argument or set of data, I can only hope that he will succumb to the implied flattery and forgive me for any accompanying exasperation.

## CHAPTER II

### THE USE OF ANIMAL EXPERIMENTS

§ 3 WORKERS in the nutritional laboratory frequently meet with criticism from the non-scientist on two rather fundamental questions. This book could have little justification were either criticism valid, and it is essential to dispose of them at the outset. Each can best be illustrated by an example, and it may be well to take the two examples from the same branch of the subject.

The first criticism, in its extreme form, is a denial that any true progress is made by laboratory experiments on small animals. "What?" it is asked, "has been learned about cod-liver oil that was not known decades, even centuries, ago?" Once it was established that cod-liver oil could cure rickets, so runs the argument—and that was discovered by physicians, not by biochemists—the expenditure of thousands of pounds, tens of thousands more likely, on salaries and equipment for laboratory scientists to try to find out how this particular substance produced this particular effect was wastefully pandering to a purely academic curiosity. It would have been much more practical and of far greater benefit to humanity if the money had been diverted into the channels of preventive and curative medicine, so that cod-liver oil could have been provided for all children suffering from, or likely to suffer from, the disease that it had been shown to prevent and cure.

§ 4 There is first of all a purely practical answer to this question. Go and ask your doctor. Does he take this view? You will find that even the least academically-minded medical man will pay at any rate lip-service to laboratory investigations; most physicians, moreover, will assert their sincere belief that this type of work has been absolutely essential to the advance of medical knowledge. And it is fairly easy to explain why they hold that view.

It is not true to say that we have known for decades, or centuries, all about the therapeutic value of cod-liver oil against rickets. What we did know was that certain doses of some cod-liver oils had been found efficacious. But there had been no certainty, not even a fifty per cent. probability, that we should strike the right dose of a particular oil for the treatment of any individual case.

True, with all except a very few unusual samples of cod-liver oil, it would probably be possible to prevent, and even to cure, rickets, provided enough were given. But that would by no means always be practicable, especially with infants and young children. You can't expect them to take an almost unlimited amount of any liquid oil, particularly not of cod-liver oil, which some people reject in even quite small doses.

It was only when the therapeutic properties of cod-liver oil had been fully investigated in the laboratory by the use of experimental animals that any really *quantitative* knowledge became available. As soon as it was established that the therapeutic property was due to the presence of a small amount of an intensely anti-rachitic (rickets-preventing or -curing) substance, subsequently to be known as vitamin D, the hunt was up. Methods were developed for comparing one sample of cod-liver oil with another, and for evaluating the anti-rachitic potency of any particular sample and, for that matter, of any other substance containing vitamin D. Today at least the physician can now buy "standardized" oils of declared potency. Once he has decided how many "units" of vitamin D—a matter we shall return to later—are required for the treatment of his patient, it is now a matter of simple arithmetic, and not a wild guess, to calculate how much of a standardized oil he must give to that patient.

§ 5 With the development of the methods of assay, in which the albino rat played, and still plays, an absolutely indispensable part, other sources of vitamin D were investigated. As a consequence, many liver oils have been examined from different fish besides the cod, and we know now of fish-liver oils having five hundred times

the anti-rachitic power of a good medicinal cod-liver oil. We also know that the vitamin is present to some extent in the fat of human and of cow's milk, and in the yolk of egg, and that it is completely absent from vegetable foods like green salads, carrots and turnips, fruits and berries, nuts, and so on; which leads to an interesting query. Why do not the children of vegetarians all die of acute rickets? To this question we shall also return later, as well as to a description of the exciting investigations that led to the production of calciferol, "crystalline vitamin D," a chemical produced in the laboratory from substances having no anti-rachitic power whatever. Though calciferol is now known to be different from the vitamin D of cod-liver oil, for reasons that we shall try to explain, it was the first vitamin to become available to medicine in the pure state; its medical value can be partly appreciated from the fact that it has for human subjects over thirty thousand times the rickets-preventing activity of good cod-liver oil.

During the war the addition of vitamin D to all margarine sold for direct human consumption was made legally compulsory. At first the required activity was that of an average good-quality butter; later the requirements were doubled. To illustrate the extraordinary potency of calciferol, the form of the vitamin exclusively used in fortifying margarine, it may be noted that to confer the necessary activity of 60 units (described later in this book) per ounce on one ton of margarine requires only one-twentieth of a gram of calciferol—that is, rather under one grain. All margarine in Britain is still "vitaminised."

The investigations leading up to the above-mentioned discoveries hang together inseparably. Had they not taken place, and had the funds so saved been used for the attempted treatment of rickets on the basis of the knowledge available prior to this undertaking, we should still be groping in near-darkness, and the potential, if not the actual, amount of rickets prevention and cure would have been immeasurably less than it in fact is at the present day. Not only have we placed the treatment



of rickets on a quantitative basis, but we have also, in so doing, unearthed—or got out of the sea—a whole collection of new anti-rachitic agents, natural and artificial. Besides that, and by no means least, we have turned a flood of light on to the cause—the etiology—and the course—the pathology—of the disease itself, valuable alike for purposes of prevention and of treatment.

§ 6 The second fundamental criticism that must be met before we can conscientiously proceed has a rather greater *prima facie* plausibility. It takes the form of admitting the scientific advances obtained in the laboratory, whether by experiments on animals or by more purely chemical and physical methods, but it asks, “What earthly reason have we for assuming that results obtained on lower animals, or even on monkeys for that matter, can be applied to man?” The answer is, though to many it may seem odd, that we have very little reason, and in any event we didn’t make the assumption! The question, in fact, shows understanding neither of the purposes for which experimental animals are used nor of the general method of applying on a large scale, whether in a factory or a hospital, results obtained in the laboratory.

§ 7 It cannot be too clearly understood that the laboratory animal, especially in nutritional investigations, plays two quite separate, though connected, parts. It serves as a reagent: in just the sense that litmus-paper is a reagent for acids, so a properly prepared rachitic rat is a reagent for vitamin D. It can be used, like a chemical reagent, not only to detect, but under suitable conditions to measure. The fact that acids turn litmus-paper red does not, of course, tell us of their ability to dissolve metals or to burn the human skin. The fact that vitamin D cures a certain experimental condition in the laboratory rat does *not* tell us that it can prevent human rickets.

That fact could only be established by experiment. But there is a world of difference between experiment and purely trial-and-error methods. The latter may not lead to any definite result, even a negative one; the former is based on already ascertained facts, applied to the problem requiring solution with due consideration

for the differing circumstances. The knowledge of rat rickets and the effect of vitamin D thereon enabled us to plan intelligently its experimental application to the human subject. The analogy drawn above—between acids and vitamin D—must not, in any event, be pressed too far. There were connections, obvious to the biochemical worker, between rat rickets and human rickets, though the etiologies of the two conditions seemed to differ. But the resemblance was sufficiently close, in pathology as well as in actual clinical symptoms, to make it most probable that vitamin D would be as effective against one as against the other.

§ 8 The first highly concentrated preparations of vitamin D were made from cod-liver oil; the process of concentration was checked at each stage by means of experiments on rats. When the chemist had got as far as he could, his material was tested on men and other animals by practitioners, medical and veterinary, respectively. It worked. But no one could have said in advance *for certain* that it would work. All one knew was that its efficacy was sufficiently probable to make its trial well worth while.

Exactly the same procedure was followed with calciferol and with its less pure predecessors, irradiated oils, irradiated cholesterol, and irradiated ergosterol.

§ 9 It is worth while mentioning here one or two other instances, including one failure, that illustrate the same methodological principles—namely, those necessary for proceeding in a scientifically sensible manner from laboratory to bedside. The fact that pure crystalline ascorbic acid, the chemical name for vitamin C, cured guinea-pig scurvy, only established a high probability that it could cure human scurvy and therefore also prevent it. Clinical trial was necessary to prove the point, and it did.

Copper is essential to the rat for the adequate formation of hæmoglobin—the red pigment of the blood. That has been proved up to the hilt. But there is even now no complete certainty that it is essential to man. Only very minute traces are required by the rat to supplement the action of the iron in its diet. One cannot

produce copper-anæmia in the human being by experimental methods, but iron to which small amounts of copper have been added, used in treating anæmia in men (and especially in children), has occasionally appeared to give better results than unsupplemented iron. It may be taken as highly probable that man needs some copper in his diet, notwithstanding that an excess has undoubted toxic effects. But so does an excess of iron itself!

§ 10 One final illustration. It has been possible to produce in rats a condition similar in many respects, at any rate superficially, to pellagra, a scourging disease affecting especially the poor maize-eating populations in tropical or sub-tropical countries.

The skin condition in rats has the characteristics of a vitamin deficiency, of which we shall have more to say later. The particular vitamin capable of preventing or curing the condition for a long time defied isolation. All that had been possible was to prepare—from yeast, for example—what the chemist calls “concentrates,” able to prevent or cure the condition in smaller amounts than the yeast from which they are made. The vitamin or “active principle” had been partly separated from the inactive yeast constituents—inactive, that is, in relation to this particular skin condition, though in fact containing other vitamins with other properties.

Experimental work of a peculiarly tricky kind made it possible to prepare from certain sources concentrates that completely cured this so-called “rat pellagra.” Would these concentrates cure human pellagra? It was by no means certain, for the etiology of human pellagra was far from clearly understood. Since the disease is a peculiarly severe one, and in very many instances fatal, it would have been an unwarrantable risk to try to effect cures with the concentrates in question, so long as any other methods of treatment were available.

This is a difficulty that has frequently turned up at a similar stage in other investigations. It must be met by indirect means. Two different facts settled the question of pellagra and entirely precluded any clinical trials of the concentrates. First, certain concentrates prepared

from maize itself had distinct curative effects on "rat pellagra." Secondly, certain foodstuffs known to mitigate or even to cure human pellagra were quite without effect on the rat condition. The conclusion was irresistible; "rat pellagra," whatever its nature, and human pellagra could not both be due solely to the absence of the same vitamin or vitamins. The problem was later solved by the discovery that nicotinic acid, a fairly simple organic compound, chemically connected with nicotine but entirely distinct in physiological properties from this poisonous base, was able to prevent or cure the main characteristic symptoms of human pellagra, as well as analagous conditions in dogs and pigs. The rat, it appeared, does not need any nicotinic acid in its diet, though it manufactures this compound in its own body for essential chemical processes in its own cells. The so-called "rat pellagra" was found to be due to dietary deficiency of other vitamins present in yeast, liver, and cereals. Their absence from human diets is rightly presumed to be most undesirable, though it does not lead to a clear-cut deficiency disease like pellagra or rickets or others mentioned in this book.

§11 The ground is now clear for consideration in a little detail of what Professor E. V. McCollum has called "the Newer Knowledge of Nutrition." I have tried to justify some of the general methods adopted both in achieving this knowledge and in applying it to human health. In so far as the arguments of anti-vivisectionists assume either of the two forms discussed above, these paragraphs may also be taken as an attempted answer. In so far as their arguments are advanced on grounds of humanitarianism—I should prefer to call it animalitarianism—they raise ethical principles that are matters of personal opinion, not susceptible of scientific evaluation. I am not concerned with them in this book.

Before we examine the main features of this newer knowledge and its application, it seems desirable to outline the fundamental facts established by what we may call "classical" nutritional science, and this is attempted in the next section.

## SECTION II—CLASSICAL NUTRITIONAL SCIENCE

### CHAPTER III

#### THE MAJOR FOOD CONSTITUENTS

§ 1 THE reader may well be already acquainted with the general features of human nutrition—the broad chemistry of carbohydrates, fats and protein, the kinds of food in which they occur and their relative amounts, the processes of breaking down—catabolism—to simpler constituents and building up again—anabolism—to the more complex substances of the living body, the two processes together constituting the *metabolism* of the organism. If so, he can skip this chapter, unless he feels that he would like a “refresher course.” The course will not, in any event, even try to cover the whole of this matter in a few thousand words. This has already been admirably done in the simplest language consistent with accuracy by several authors, especially by Dr. W. R. Aykroyd, Dr. Leslie Harris, Professor V. H. Mottram, Mrs. Barbara Callow and Dr. T. R. Parsons.

Nevertheless, for the benefit of those who have forgotten the salient facts, a *résumé* seems called for. It must of necessity contain a number of statements that will appear platitudinous, but I shall hope to make up for that by a certain scepticism that will not escape the notice of those who manage to read beyond this chapter. Nor is it, to my mind, any sign of intellectual distinction to be for ever on guard against platitudes where these are relevant to an argument that may not be so obvious in its conclusions.

§ 2 The ingestion of food by the human or any other animal fulfils two main purposes. First, it has to supply the energy that is ultimately expressed either as the heat of the body, when this has a temperature higher than that of the surrounding medium, or as movement of various limbs and organs, whether that movement is con-

trolled at will, like the movements of arms and legs, or is partly involuntary, like that of the eyelid and lung, or is wholly involuntary, like that of the heart. Secondly, it has to supply the tissues with fresh material to replace what is worn away or wasted in any manner, including the depots of energy-giving substances, where these may have been temporarily depleted, as during illness, fasting, pregnancy or lactation.

§ 3 There is one sense in which the second purpose is more important than the first, quite apart from the fact that the child or other young growing organism needs not merely to replace tissues, but actually to build them up. The main food constituent required for this purpose is the nitrogen-containing protein. Without it life must cease, for the breakdown of muscle tissue and losses of glandular secretions and mucus from the intestine, all consisting largely of protein, occurs continuously during movement of any limb or organ, including the movement of heart and lungs even during sleep. The breakdown products are not retained in the system and built up again; they are *excreted* from the body, mainly in the urine through the kidneys, though it is frequently forgotten that hair and the nails of fingers and toes are, speaking physiologically, also excreta. Moreover, *fæces* are to a considerable extent not really excreta at all, because they include food that has never been digested, or parts of food that have not.\* The *fæces* also contain a large amount of bacterial substances, dead and alive.

This continuous excretion of nitrogenous matter means that the food *must* contain sufficient protein to replace the various losses, including any tissue broken down during muscular movement; otherwise the muscles could not continue functioning, and without movement there is no animal (or vegetable) life.

Special techniques devised during the last few years

\* C. M. Thomas always used to tell the boys in his biology class at St. Paul's School that the alimentary canal, from the mouth through the gastro-intestinal tract to the anus, is not *inside* the body at all, a statement that is embryologically if not physiologically true.

have given abundant proof of what has for some time been suspected on various grounds. There is, it is now clear, no such thing as a state of rest in the living body, even if this body is completely quiescent, as in deep sleep. The analogy with the internal-combustion engine, frequently employed in descriptions of the difference between nutrients supplying energy and nutrients contributing to body structure, breaks down at this point. In the motor-car the machine parts, chassis and body, once made, last a long time: the car has to be fed with petrol and lubricant, but not with steel and plastics. Not so the animal body—or the vegetable body, for that matter. As long as there is life, there is flux. Our apparently fixed and stable muscles, even our rigid bones and teeth, are continuously losing substance, whether they appear to be engaged in active work or not, and this loss has continually to be made good. An animal receiving no lime in its diet will continue to lose lime from its bones, teeth and tissues generally. On a diet containing inadequate protein—the body's source of the element nitrogen—more nitrogen will be lost in urine and faeces than is ingested in the food. The body is said to be in "negative nitrogen balance." The losses from this continuous breakdown of body protein, independent as they are of muscular activity, are in point of fact far greater than any superimposed losses due to muscular activity, and this has important consequences in connection with individual food requirements.

§ 4 Fats and carbohydrates are the normal fuels of the body, supplying the energy necessary for the two purposes already mentioned. Fats, particularly, are good fuels, because per unit of weight they give out more heat when burnt up than do carbohydrates. But carbohydrates will do very nicely, and a fat-free diet does not involve metabolic nihilism. A carbohydrate-free diet is also capable of supporting life for some time, but is not at all palatable to dwellers in temperate, sub-tropical or tropical climates. In the polar regions, however, it is almost the normal regime; seal meat, for example, consists entirely of fat and protein.

Further, the proteins themselves are perfectly good sources of energy, if rather expensive ones. They give out, when burnt, the same amount of heat as carbohydrates, and they are equally good fuel. A diet of protein alone, however, though it is calorifically possible, is physiologically abnormal. One of the main reasons is that the kidney has to go out of its way to develop the capacity to deal with the extra amount of protein breakdown products, if protein should be supplied in sufficient quantity to provide much of the fuel as well as tissue matter. There are, however, some native tribes that live on a meat and milk diet with an unusually high protein content, though the meat and milk supply fat and carbohydrates as well.

§ 5 This distinction between protein on the one hand and fat and carbohydrate on the other is, nevertheless, of very much more than theoretical interest. When we come to consider the different chemical compositions of the diets eaten by rich and poor, we shall find that it has a very practical implication. The rich are never likely to suffer from calorie shortage, however much their diet may topple over on the side of protein excess, with a possible danger of kidney trouble; the poor, on the other hand, whose diet is overloaded with the cheaper foods that consist largely of carbohydrates, live permanently on the borders or within the territory of protein starvation.

§ 6 It is now necessary to consider the chemistry of fats, carbohydrates and proteins, in so far as it bears on their main functions in nutrition. Although the proteins, by virtue of their unique importance, perhaps have claims to be considered first, their highly complex nature makes it advisable to take them last, and we will begin with the carbohydrates.



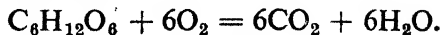
## CHAPTER IV

### CARBOHYDRATE CHEMISTRY

§ 7 Much the best known of all the chemically pure carbohydrates is sucrose or cane-sugar (sometimes called saccharose, but not therefore on any account to be confused with the synthetic sweetening agent, saccharin, a coal-tar derivative). Nevertheless, sucrose is not the simplest carbohydrate known even to the layman. Glucose, or dextrose, which also bears the name grape-sugar, is now so much in vogue, both as a subsidiary foodstuff and as a substance for medicinal use, that many people must have asked their chemist friends exactly in what way it differs from cane-sugar. It is obviously much less sweet and also picks up moisture and "cakes" a good deal more easily. As these chemists would put it, it is more "hygroscopic" than sucrose.

§ 8 Glucose is, indeed, a different substance from sucrose, but not, we shall shortly see, quite as different as chalk from cheese! Its chemical formula is  $C_6H_{12}O_6$ ; this shows one of the characteristics of all carbohydrates: the presence in the molecule of two hydrogen atoms to every one oxygen atom. That is the ratio of hydrogen to oxygen in water; were it not for the fact that to do so would be to represent quite falsely the way the atoms of carbon, hydrogen and oxygen in glucose (and other carbohydrates) are linked together, we might write the formula of glucose as  $C_6(H_2O)_6$ .

§ 9 It should be noted here that the molecule of glucose needs for its complete combustion to carbon dioxide gas and water, whether in a crucible or in the human body, only just sufficient oxygen to combine with the carbon, for it already itself contains enough oxygen to combine with all the hydrogen present. Put in the form of the equation used by chemists, we write this combustion of glucose



This equation, besides describing the products that take part in the reaction of combustion, glucose and oxygen, as well as those that result from it, also gives in concise form the chemist's knowledge of the quantities involved. It comprises the statement that 180 parts by weight of glucose require 192 parts by weight of oxygen for complete combustion to 264 parts of carbon dioxide and 108 parts by weight of water.

§ 10 Glucose is by a long way the most important carbohydrate known to the nutritionist. It is the form into which almost all carbohydrate is broken down or transformed in the human organism prior to its circulation in the blood-stream, by which route it reaches the muscular tissues, there to be burnt up. In the process of being burnt it liberates energy, either the energy of movement—kinetic energy—exhibited by the muscles, or the energy of heat—thermal energy—that maintains the body temperature.

§ 11 If two molecules of glucose were to combine together and to lose a molecule of water in the process, we should get a compound that might be regarded as a double sugar, especially if it were possible, by adding a molecule of water to it, again to break it down to two molecules of glucose. The two processes are known to chemists as condensation and hydrolysis respectively, although the term condensation is also used rather more widely to cover a number of similar chemical reactions. But this combination of two molecules of sugars of the  $C_6$  class—the monose class—to form one of the  $C_{12}$  class—the diose class—in fact takes place during the normal anabolism of plants and animals; similarly the hydrolysis of dioses to monoses is a catabolic process of primary importance to both.

§ 12 The diose formed when two molecules of glucose are condensed is the chief sugar of malt-extract, maltose. Glucose itself is the most easily assimilable of all sugars, for it requires from the organism no catabolic processes before it can pass into the circulation. Further, it is completely utilized by the healthy body. Thus, as a source of energy it is doubly economical. Although

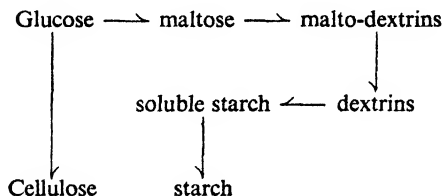
maltose has to be hydrolysed in any organism that feeds on it, it also is a highly economical carbohydrate, for it breaks down into glucose only and is therefore completely available as fuel. When maltose molecules are joined together by a further series of condensations, more complex carbohydrates are formed. As they become more complex, they lose their soluble, sweet crystalline properties and acquire gummy or even insoluble characteristics. There is, in the terminology of the physical chemist, a change from crystalloidal to colloidal compounds (from the Greek *κόλλα*, glue). Furthermore, the possibilities of variety in the resulting condensation products also rise rapidly with the number of condensed molecules. They can, as it were, arrange themselves in long chains, in spirals, in networks, and so on.

§ 13 There comes a point where the condensation product is so complex, colloidal and insoluble that it ceases to be available as a food for man. Cellulose is the material of which the cell-walls are made in plants; it can be broken down by the chemist's reagents to monoses, mostly glucose, but the chemist's stomach cannot do this. On the other hand, the bovine stomach can; in this respect the cow seems a better man than the chemist. Starch, on the other hand, which is also primarily a glucose condensation product, is hydrolysed by adult man, but his infantile offspring only gradually acquires the talent.

§ 14 The preparation of a malt extract consists in dissolving out of germinating barley all the material soluble in water, and then concentrating this material to a thick paste. The soluble constituents of malt extract are almost all derived from the starch in the germinating grain; the necessary catabolic process is furthered by the presence in the grain of certain natural ferments or enzymes, some of which may still be present in the finished malt extract itself. The rôle of enzymes in plant and animal metabolism has by now been studied for decades by thousands of biochemists. The human stomach, intestines and other digestive organs (such as

pancreas and liver) are also producers of enzymes, among whose most important rôles is that of stimulating—"catalysing" the chemist calls it—processes of hydrolysis exactly comparable with the breakdown of starch in germinating barley. Bacteria also are great enzyme-producers; certain of these micro-organisms produce enzymes that can hydrolyse cellulose, and these bacterial enzymes are responsible for the breakdown of cellulose that occurs in the digestive tract of cattle and other ruminants, enabling them to use "roughage" as food.

We may here note down, in order of increasing complexity, the main condensation products of glucose—



§ 15 In § 13 above there occurred the phrase "monoses, mostly glucose." We must now return to the complications implied in that phrase. The truth of the matter is that glucose is by no means the only monose; indeed, you could have guessed that, for there would have been no need to give a class-name—monose—to a substance—glucose—if there had been no other substances in the class. And there are a fairly large number known, though fortunately only two more of them are of primary importance in human nutrition. These are *lævulose* (fructose), or fruit sugar, and galactose. Neither of these monoses occurs as such to any important extent in nature; each can combine with glucose, the first to form ordinary cane-sugar and the second lactose, or milk-sugar, a diose present only in milk, but apparently in that of all mammals.

These two dioses are, as sources of energy, every bit as good as maltose, speaking chemically, for they contain exactly the same proportions of carbon, hydrogen and

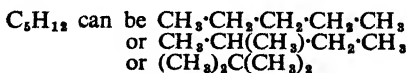
oxygen.\* Whether this is also true of them in a physiological sense is another matter. The chief reason for thinking that they are is to be found in the fact that diabetics, to whom sugar is literally a poison, seem to be no better able to take sucrose than glucose and little better able to take lactose. This sugar appears to be specially suited to the infant digestive system, though the normal adult is also able to utilize it perfectly well. It is very much less sweet than sucrose, and so is glucose. The extreme sweetness of cane-sugar must be associated with the l  vulose half of its composition and has its disadvantages; excessive intake may lead to nausea.

Quite apart, therefore, from any slight physiological inferiority of sucrose, arising from the fact that it must be hydrolysed to glucose and fructose before it leaves the intestine, maltose, or, even better, glucose, can claim to be the carbohydrate of choice for supplying energy before, during, or after an emergency. This is the main reason for the medicinal use of glucose and has also suggested its use by athletes and others immediately before they take violent exercise. There is, however, some evidence to suggest that the fructose half of sucrose is every bit as well utilized as glucose itself, while fructose that has been previously separated is absorbed only about half as fast as glucose. Here possibly we have again an instance of small changes in chemical structure

\* If some inquiring reader asks how there can be a number of *different* compounds, having the *same* composition, I shall be reduced to saying, "That's chemistry, that was." Compounds differ from each other not only in the number of atoms of different elements they contain, but also in the way those atoms are joined together in space. This is one of the complications that make chemistry so fascinating a subject. The more complex the molecule, obviously the more opportunities for it to have "isomers," as they are called. Organic chemistry is concerned not merely with the atomic alphabet, but also with the molecular anagrams built up from it. That is why, for example,



while



and so on.

associated with marked differences in physiological properties. For both forms of fructose are identical in chemical composition—that is, in their relative proportions of carbon, hydrogen, and oxygen.\* Anyhow, the greater sweetness of sucrose may well make it for rapid bodily stoking a less useful fuel than glucose.

\* See also footnote to p. 18.

## CHAPTER V

### CARBOHYDRATES IN THE BODY

§ 16 INGESTED glucose and other utilizable carbohydrates are carried to the tissues and chiefly to the liver, where the non-glucose monoses are changed to glucose and condensed, as is glucose itself, to *glycogen* or "animal starch." This, though differing in many respects from vegetable starch, is its animal analogue. It can be drawn on by the normal body whenever supplies of fuel are wanted. The glycogen is simply broken down to glucose, which passes out into the circulation. Diabetic patients are those in whom insulin, an internal secretion of the pancreas that partly controls the balance between glucose and glycogen, is deficient or absent. Insulin favours glycogen formation or storage and inhibits the production of glucose; adrenaline, the internal secretion of the adrenal gland, acts in the opposite direction. This is a scientifically recognized genuine instance of "endocrine balance"—a phrase so much the stand-by of those who attempt to grow rich at the expense of credulous hypochondriacs as to be more honoured in the avoidance than the use.

§ 17 While outlining the chemistry of the carbohydrates in food, it has been possible to give some idea of their break-down or catabolism in the body of the eater. This particular form of break-down is called hydrolysis, and is effected by the enzymes secreted in the saliva and by the juices of the digestive organs, the stomach and intestine, the pancreatic gland that pours its secretions into the gastro-intestinal tract, and the liver, whence the bile flows, a supply being kept stored in the gall-bladder. These enzymes together hydrolyse not only sugars, but, as we shall shortly see, also the fats and proteins to simpler substances that, by virtue of their smaller molecules, can then pass through the cells of the intestinal wall into the blood-stream.

The anabolism of the monoses to glycogen is a process that has not been found easy to investigate, but it is fairly well established that it takes place extensively in the liver, though it must also occur in the muscles themselves. Since glycogen is almost completely insoluble in blood plasma, it cannot be conveyed as such to the muscle fibres, of which it is an essential constituent. It is the oxidative breakdown of glycogen to carbon dioxide and water, *via* lactic acid and other "intermediates of carbohydrate metabolism," as the physiologists call them, that furnishes the energy necessary for muscular activity.

The muscle-cells, like those of the liver, are therefore able both to build up glycogen from glucose (and other monoses) and to break down glycogen to simpler substances.

§ 18 It is well known that carbohydrate can be turned into fat in the body. Fats are chemically very different from carbohydrates, and it therefore becomes pertinent to ask how, and where, this change is effected. Here again it seems certain that the carbohydrates must first be made available as simple monoses, and it is almost equally certain that these undergo degradation to still simpler substances—pyruvic acid is the name of one of them—that are then synthesized into fats. Exactly where this building up takes place seems to be a much more difficult question to answer, and it also seems likely that the needs of the body for fat cannot be indefinitely met by the conversion of carbohydrates.

The special sugar secreted in milk is wholly synthesized in the mammary gland, which has the unique ability to convert some of its supplied glucose to galactose; this is then condensed, molecule for molecule, with more glucose, to give the sugar—lactose—characteristic of milk alone.

§ 19 Although we know as much about the metabolism of carbohydrates as of any other food constituent, it is true to say that physiologists have yet to find an answer to many questions that the nutritional practitioner may, and does, put to them. Indeed, some dietary habits that are well observed (and have been well



observed!) seem incapable of explanation in the light of existing laboratory knowledge.

In Chapter IV we noted that the amount of oxygen needed to burn carbohydrates is precisely known. So is the amount of heat given out during the burning. For practical purposes it is accurate enough to remember that every gram of carbohydrate is the potential source of 4 large Calories, or the ounce of 116. Those are the quantities of heat given out by the stated amounts when they are burned in a laboratory instrument, called a bomb calorimeter, in which the amount of heat liberated can be very exactly measured. Does this mean that every gram of carbohydrate eaten by a man will contribute the same amount of heat either as heat or as movement or as both?

It certainly cannot mean that, without qualification. We have already seen that glucose and cellulose are both carbohydrates, and it is as near as no matter correct to say that equal weights of each will liberate the same amount of heat when they are burnt in the bomb calorimeter. Yet it is perfectly obvious that a man does *not* obtain the same amount of nourishment from a pound of blotting-paper as from a pound of barley-sugar. The first consists of almost pure cellulose, but it is not digested at all, though it is a potential source of one hundred per cent. glucose, speaking chemically; the second, apart from the water and flavouring matter it contains, is fully utilized as a carbohydrate—that is, up to the whole of its glucose content. It is clear that digestibility may modify energy value. Is it certain, then, that all forms of digestible carbohydrate, even of the three utilizable monoses, dextrose, lævulose and galactose, are equally useful food-substances?

§20 Again, there is an impression among some nutritionists that different forms of starch may have somewhat different nutritive values. Particularly does it seem customary to make some kind of distinction between the starch of seeds, of which wheat, maize (yellow corn) and rice are the most important to the human race, and that of tubers, notably the potato.

The starch of green leaves may also possibly have yet another, somewhat different, evaluation.

Another unanswered problem is presented by toast. It is quite generally believed that toast and the fashionable forms of breakfast rye-biscuits, as well as other biscuits made without fat, and also the cooked breakfast cereals originating in the United States, are less fattening than bread, weight for weight of carbohydrate. Yet it is also recognized that toast and many other forms of cooked starch are more easily digested than bread, especially new bread. The process of cooking effects a partial breakdown of the starch to dextrins similar to those produced from barley starch during malting, and one can appreciate that this preliminary hydrolysis may relieve the human enzymes of some work and facilitate utilization by the body of starch that has been so treated. But it is difficult to understand why the more easily assimilated forms should also be the less fattening! The practice of consuming various forms of breakfast cereal and rye-flour biscuit, as a less fattening alternative to white bread, seems to have a mystico-economic origin rather than an experimental one, except that these products may contain indigestible roughage of a "branny" nature which can temporarily satisfy hunger, though it can contribute little if anything to the body's energy requirements.

§21 As far, therefore, as experimental knowledge goes—knowledge based both upon chemical and biochemical laboratory work and also upon clinical observation—we cannot discriminate between the calorific values of different forms of carbohydrate, except to the extent that some forms are partly or wholly undigested. The most easily assimilated forms may, however, have advantages under certain conditions, either because their catabolism requires less digestive activity or because its speed will make more rapidly available the energy required for some special emergency.

## CHAPTER VI

### THE CHEMISTRY OF FATS

§ 22 WE have seen that the chemistry of carbohydrates, as used by the human body for energy production, reduces itself to a consideration of three monoses only, of which one is far more important than the other two. The story of the fats is more complicated. Fortunately, however, we can begin with certain eliminations.

Books on chemistry will be found often to refer indiscriminatingly to *fats* and to *oils*, and yet at other times to imply that they are quite different substances. This apparent confusion can be explained in terms of the history of chemistry and is purely a verbal affair; the chemist is quite clear as to what he means by the words. We can become equally clear if we briefly consider the three different classes of oils known to science.

§ 23 First, we have the fatty oils which occur as indispensable parts of plant and animal tissues. They constitute, as we have already seen, the richest "fuel" for living matter. The term "oil" is generally applied to those that are liquid at ordinary temperatures (say down to about 70° F.), such as olive oil, linseed oil, cod-liver oil, castor oil, while the term "fat" is used for those that are solid at this temperature—butter-fat, for example, and mutton-fat. These solid fats, however, have often also been given some alternative or descriptive name, so that you find in the group lard, cocoa-butter and suet. We shall return to the chemistry of these natural fats and oils in rather more detail.

§ 24 The two other classes of oil are the mineral oils and the essential oils. The great group of mineral oils (petroleum, paraffin, vaseline) all come in the chemical category of hydrocarbons, with benzene and naphthalene. They contain, that is to say, hydrogen and carbon only; whether they are found native, as in the great oilfields, or made by the factory treatment of coal, they are the

natural or the artificial degradation products of past or present plant and animal tissues. Though certain complex and rare hydrocarbons are indeed found in living tissues—for example, in the leaves of certain plants and the livers of some species of shark—they occur in relatively small quantities. At present only one hydrocarbon is known to play an essential part in human nutrition, and that is carotene. Since this deep orange-red pigment is converted by the animal body into vitamin A, it will be convenient to postpone any more detailed reference until we consider the vitamins themselves.

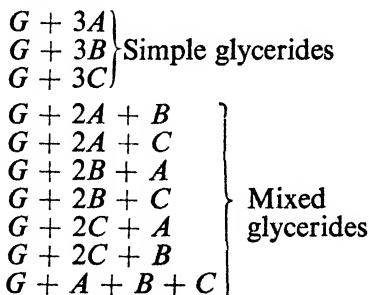
We must also dismiss with a glance the important group of ethereal oils or essences, extracted from the flower or fruit of plants, or synthesized by the chemist. Familiar examples are otto of rose, oil of lemon, eucalyptus oil. Although these oils contain oxygen as well as carbon and hydrogen, their chemical structure and properties are entirely different from those of the fatty oils. Some of them may have a minor, though important, dietetic function in stimulating appetite and gastric secretion.

§ 25 It is not known how hydrocarbons or essential oils are metabolized in the body, if indeed they are metabolized at all. Liquid or semi-solid petroleum (“vaseline”) passes with little absorption or loss straight through the alimentary canal; on the other hand, the essential oils may well be subject to some process of digestion and absorption. In any event, the fuel contribution made by hydrocarbons or essential oils would be almost nothing, even if their carbon and hydrogen were completely burned to supply energy to the body, since they are present in ordinary foodstuffs only to a negligible extent.

§ 26 The oils and fats with which we are here directly concerned, then, are only those that can legitimately be described as edible—at least in small quantities. (We can consume castor oil, for example, only in limited amounts, but still it is edible.) These fats and oils are, in short, substances of natural origin, invariably present to a greater or less extent in living organisms; they

contain carbon and hydrogen and a proportion of oxygen much smaller than that of the carbohydrates, but also as an integral part of their structure. These substances possess the fundamental chemical property of *saponifiability*, which simply means that they can be decomposed by alkalis (soda, potash, lime) to give glycerol and the corresponding *soap*.

The soaps are compounds of alkali and fatty acids; glycerin (or glycerol, to use its more strictly scientific name) is an alcohol. The fatty acids and the alcohols are two highly important classes of organic substance. The simplest representatives of each class are, respectively, methyl alcohol (wood spirit) and formic acid (present in the sting of the ant). The next simplest representatives are more familiar, ethyl alcohol (ordinary alcohol) and acetic acid (the acid of vinegar). Very many fatty acids and alcohols are known, but it is not always realized by the non-chemist that glycerol is an alcohol; it shares with the other members of its class the property of combining with organic acids to form esters. The esters of glycerin with organic acids are called *glycerides*; theoretically an enormous number of them is possible. First of all, every molecule of glycerol can combine with three separate molecules of fatty acid, which may be the same acid or different acids. Schematically, all the following various *glycerides* are possible, where glycerol is represented by *G* and fatty acids by *A*, *B* and *C*.

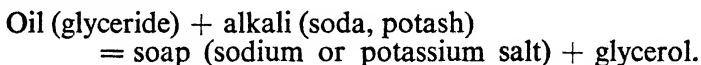


The number of fatty acids known is very considerable—probably at least a hundred occur in nature, though not

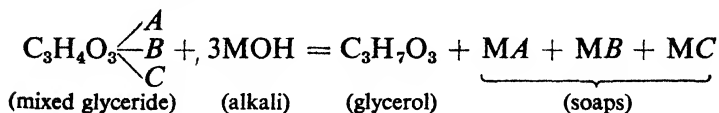
more than a dozen or so occur in sufficient quantity to be of dietary importance.

§ 27 The oils, then, are made up mainly of fatty acids and glycerol; they consist of anything up to 99.5 per cent. of mixtures of glycerides, which may themselves be simple or mixed glycerides. The extraordinary range of properties shown by these oils is an expression of the immense number of glycerides theoretically possible and practically encountered. Some oils consist of one nearly pure simple glyceride, such as olive oil, which is mainly the simple glyceride of oleic acid; others, such as cod-liver oil, are highly complicated mixtures of glycerides, containing fatty acids that have not individually been completely identified or even isolated.

But all these oils share in common their susceptibility to the action of alkalis. If they are treated, under suitable conditions, with soda, the glycerol splits off from the fatty acids, which themselves combine with the soda to form their sodium salts. The salts formed by the combination of alkali with fatty acids are simply soaps, the sodium salts being hard soaps and the potassium salts soft soaps. The simple facts of saponification can be expressed thus:—



In chemical symbols the matter is put even more tersely:—



§ 28 Until quite recently, even in so authoritative a work as the *Encyclopædia Britannica*, oils were defined as naturally occurring mixtures of glycerides of the fatty acids; such mixtures could themselves be defined strictly in terms of their chemical composition. Nevertheless, it is true to say that if a chemist ever found in nature an oil which strictly conformed to this definition, he would have the shock of his life, for no natural

oil does conform to it. Later editions of the *Encyclopædia Britannica* have avoided this paradox by re-defining the oils so as to indicate that they contain something else besides glycerides.

When an oil has been saponified it is possible to separate from the resultant mixture of soap and glycerol a portion of the original oil, generally between 1 and 2 per cent., that, unlike the soap and glycerol, is insoluble in water, though it shows a limited solubility in soap solution. If this material is again treated with alkali, it undergoes no appreciable further change. It cannot be converted into soap. It is, in fact, *unsaponifiable*. Until fairly recently the unsaponifiable matter in an oil was regarded, if only tacitly, rather as an accidental and almost reprehensible impurity in what, according to the text-books, was a naturally occurring mixture of glycerides. A certain amount of purely academic research had been applied to examining this intruder, and some members of an interesting group of substances, called sterols, had been found almost invariably to be present. These sterols, of which we shall have more to say later, are well-marked white crystalline substances, showing some of the properties both of complex hydrocarbons and of alcohols. They can, by appropriate means, be made to form esters with acids, and they show certain interesting colour reactions and optical properties. One of them, cholesterol, invariably found in the unsaponifiable matter of animal fat, is also found in blood, in brain and in bile (whence its name, from the Greek *χολή*, bile). Some common types of gall-stone consist of almost solid cholesterol. Besides the sterols in unsaponifiable matter, there was also found a liquid, a yellowish heavy oil, about the chemistry of which practically nothing was known.

The unsaponifiable matter of oils suddenly assumed an entirely unexpected importance. It was found that any fat-soluble vitamins present in the oil could be completely separated with the liquid part of the unsaponifiable matter, and it became at once obvious that this portion of the oil, hitherto rather despised and rejected, must

have a profound physiological significance. Although it might amount to little more than one-half per cent. of the oil, it contained what was, from certain aspects, the oil's most interesting constituents.

§ 29 From the point of view of fat metabolism, however, which we shall consider in the next chapter, there is one more fact of outstanding importance to note. Fortunately, it is a fact with which everyone is acquainted, consciously or subconsciously.

If your hands are covered with grease, using that term in a vague and non-chemical sense to include such diverse substances as butter, margarine, olive oil, kerosene, lubricating oil—for they are all alike in this respect—you can't clean them with water. Fats and oils—all of them—are almost completely insoluble in water. But they can be persuaded to disperse themselves in a watery medium if certain substances are dissolved in the water. These substances belong to the general class of colloids, to which we have already had occasion to refer. Not all colloids act as dispersing agents, but it is broadly true to say that all the dispersing agents of physiological importance, and many others, are colloids.

The fat (milk-fat or butter-fat) of milk and cream is kept dispersed by virtue of the colloids present, the proteins caseinogen and lactalbumin. When you wash your hands with soap, it is the colloidal soap that disperses the grease and allows it to be washed away as finely divided globules suspended in the solution. Other colloidal dispersing agents belong to the group of gums, of which gum arabic (gum acacia) finds considerable use in the preparation of pharmaceutical emulsions.

Reference must here be made to an important and universally distributed class of substances allied to the fats, but containing phosphorus and nitrogen as well. They are referred to under the name of phospho-lipids; their exact rôle is not clearly understood, but they almost certainly play an essential part in fat metabolism. Their amount, however, is relatively small, and they cannot contribute materially to the body's calorific requirements.



§ 30 For digestive purposes, the salts of certain acids secreted by the gall-bladder are of outstanding importance for emulsification. Curiously, and significantly, these acids show a close chemical relationship with the sterols, to which we have already referred as important constituents of the unsaponifiable part of fats. The sterols themselves, however, owing to their insolubility in water, are of no physiological value as emulsifying agents. But without their relatives, the bile salts, there could be no digestion of fat. When the sterol-bile acid relationship is disturbed with the formation of gall-stones, which consist almost entirely of crystalline sterols, there is a relative shortage of bile-salts, owing to purely physical obstruction, but it is possible that there is also an absolute loss due to the accumulation as sterols of material that should have gone to make bile acids.

§ 31 To summarize this chapter, which may perhaps appear to some extent a digression, the fats must be remembered as water-insoluble mixtures of glycerides and unsaponifiable matter, the glycerides being capable of saponification into the salts of fatty acids and glycerol, both of which are soluble in water. The fats, further, contain only carbon, hydrogen and oxygen (like the carbohydrates), but the amount of oxygen is much less than that of carbohydrates, and to this they owe their high calorific value. As a matter of interest we give below the relative *percentage* amounts of the three elements in glucose the glyceride of oleic acid—olein, which can be taken as a fair average for fats in general.

	Carbon per cent.	Hydrogen per cent.	Oxygen per cent.
Glucose . . . .	40.0	6.7	53.3
Olein . . . . .	77.4	11.7	10.9

## CHAPTER VII

### FATS IN THE BODY

§ 32 It is a curious and rather disconcerting fact that the precise nature of fat catabolism seems still not to be fully understood. We know that the pancreas and the intestine secrete enzymes, called lipases, that can break down fats in a manner similar to the action of alkalis, described in the last chapter. The acid nature of the stomach juices is not very favourable to fat hydrolysis. The lipases are known also as lipolytic or, better, lipoclastic enzymes; like all enzymes, they act most energetically at a characteristic optimum acidity or alkalinity. The main site of fat digestion is the intestine. The action of the intestinal lipases is reinforced first by the entering pancreatic juice, which contains a specific lipase, called steapsin, and secondly by the simultaneously entering bile, which is alkaline.

§ 33 It is possible, and most text-books of physiology throw no doubt on the possibility, that all fat entering the intestine is broken down to glycerol and the salts of fatty acids. These salts, being soaps, are emulsifying agents and themselves assist the dispersing action of the bile salts, so that any unhydrolysed fat is present as a fine emulsion, a state that facilitates further hydrolysis. Lipase, alkali, bile-acids, soap—all appear to co-operate in the intestinal hydrolysis of fats. The finely emulsified fat suspension is, according to the conventional view, in the end entirely converted to a mixture of soaps and glycerol. These substances, being soluble in watery solutions, can pass through the walls of the intestine; they then reach a special series of ducts, called the lacteals, and in the process re-combine to a fine emulsion of fat. This fat is conveyed to the various depots of fat; one of the most important is situated in the abdomen and is called the *receptaculum chyli*.

§ 34 There are certain facts that make it difficult to accept this fairly simple account of fat-breakdown

by hydrolysis, and subsequent re-formation. Most of the ingested fat—all except about 10 per cent.—can be traced in the blood-stream, the main thoracic duct, and the major lymphatics, apart from what can be found unchanged in the faeces.

It has, however, been experimentally demonstrated frequently that the chemical composition of an animal's body-fat can be materially altered by changing the nature of its food-fat: the composition of milk-fat may be similarly affected. On general grounds, and by analogy with protein metabolism, one would expect the body to take advantage of *complete* fat hydrolysis by selecting from the liberated fatty acids pretty exactly those required, and in the necessary proportions, to build some fairly constant body-fat, specific to the fat-eating animal.

§ 35 A different theory is gaining increasing acceptance among physiologists; it overcomes the last-mentioned difficulty, though it throws little light on the 10 per cent. intestinal loss of fat. It is that fat passes largely unhydrolysed, in an emulsified state, direct into the blood-stream, the lipases having the function of assisting only sufficient hydrolysis to give the amount of soap necessary to co-operate with the bile-salts in producing a fat emulsion fine enough to pass through the walls of the intestinal cells and out into the circulation. The work of Professor Alastair Frazer and his colleagues suggests very strongly that fat is in fact absorbed by both the lymphatics and the blood-stream, but we have as yet little information as to how much goes by each route and what determines the amounts.

The reader may consider this conflict of view an interesting enough academic controversy, but of little practical importance to the nutritionist. When, however, we come to survey the fat-soluble vitamins, and particularly the question of their storage and availability in the body, it will be seen that the gaps in our knowledge of fat-digestion may have a very direct bearing on some problems of vitamin deficiency.

However that may be, it is important to note in passing that certain fats, in particular milk-fat and the fatty

substances in green leaves, as well as special fats like cod-liver oil, differ from the carbohydrates in that they are not simply fuel. They are also carriers of such fat-soluble vitamins\* and pro-vitamins as are present in their unsaponifiable matter. Physiologically speaking, therefore, as well as chemically, the fats must be regarded not simply as a mixture of glycerides, but as glycerides *plus* unsaponifiable matter.

§ 36 Apart from the relatively inefficient nature of carbohydrates as a body-fuel, at any rate in comparison with fats, they appear to be excellent sources of raw material for fat-building. It is not known for how long a man can live on protein and carbohydrate alone, assuming that he gets enough for his calorific needs and that his fat-soluble vitamins are provided separately. But it is quite certain that a man can "put on fat" far in excess of the amount of fat in his food. Indeed, it is arguable that a protein-fat diet might be less fattening than a protein-carbohydrate diet, for the lower calorific value of carbohydrates would necessitate larger rations of the latter.

§ 37 On the other hand, carbohydrates seem, at any rate over prolonged periods, essential for the utilization of fats. Even the Esquimaux, I am told, consume considerable quantities of carbohydrates in the marine plants that they eat as a supplement to seal-meat and bear-meat. A scientific study of native diets in polar regions might throw light on interesting metabolic problems.

One of the oldest tags of physiology is the statement that "fat is burnt in the fire of carbohydrates." As this is a statement that no chemist has ever succeeded in understanding, I cannot help feeling that it says nothing more than is said in the first sentence of the last paragraph.\* But no book on nutrition would be complete without it.

§ 38 The fats of the body depots are conveyed by the blood-stream, of course in a finely emulsified state, to the various muscles, where they are gradually broken down to simpler substances and finally to carbon dioxide

\* Some recent work indicates that it isn't true even in this sense.

and water; calories are released at every stage, until the full quota of 9 calories per gram of fat has been given up either as heat or as energy of movement.

In certain pathological conditions, notably in diabetes, the body has lost the power to carry fat oxidation to completion. Various products of this incomplete combustion, known as "acetone bodies," but more accurately described as ketones and keto-acids, are released into the blood-stream; this condition is called *ketosis* (sometimes, more loosely, *acidosis*), and, since these ketones are poisonous when they circulate freely in the body, the condition needs prompt attention. The administration of insulin to the diabetic causes his carbohydrate metabolism to return towards normal, and with the renewed utilization of the carbohydrates, and the disappearance of glucose from blood-stream and urine, comes the ability to complete fat-combustion and prevent the circulation of ketones. Other mild conditions of ketosis, such as that associated with excessive vomiting, can sometimes be remedied by simply administering glucose, either by mouth or by injection. It is facts of this kind that are, no doubt, behind the above-quoted apothegm so baffling to all but certain physiologists. It is certainly true that the human body finds it very difficult, if not impossible, to cope with excessive quantities of fat in the absence of carbohydrates. It seems likely, however, that a ration very carefully balanced between fat and protein, which has some of the "fat-burning" properties of carbohydrates, could be taken for a considerable period without serious ill-effects.

§ 39 Whether different fats, by virtue of their different chemical compositions, have different nutritive values, apart from any differences in digestibility, is a question that leaps to the mind. All fats are taken as giving up (if completely utilized) 9 calories per gram. Yet any chemist can calculate that the fats containing a high proportion of the lower fatty acids (those, that is, with fewer carbon atoms in the molecule) must, by virtue of their relatively higher oxygen contents, have lower calorific values; they are, as combustible substances,

intermediate between the carbohydrates and the fats that contain high proportions of the higher fatty acids. Coconut-oil and butter-fat should be worse fuels, gram for gram, and if fully burnt in the body, than mutton-fat and margarine. The authorities appear silent on this matter.

↳ The contrast with carbohydrates is rather striking. *All* carbohydrates are, as near as no matter, of equal calorific value to the chemist; in food they range from the rapidly and directly absorbed to the completely indigestible. The fats, on the other hand, have detectably different calorific values, yet by the practical as well as by the theoretical physiologist they seem to be treated as effectually interchangeable. It must, however, be observed that there are some exceptions. Certain fats and oils are of little value as foodstuffs (that is, as sources of energy), because their intake is limited by the presence of unpalatable constituents—as in fish-liver oils, rancid fats of all kinds, and so on—or of actually irritant or poisonous substances—as in castor oil or croton oil, though these last may be used in small doses for medicinal purposes.

## CHAPTER VIII

### THE CHEMISTRY OF PROTEINS

§40 EVERY cell of the animal or vegetable organism consists to a greater or less extent of "protoplasm." This is a viscous liquid of colloidal nature and consists of a delicately balanced mixture of all those substances that go to make up "living matter." First of all these in importance are the proteins. Of the three major groups of food-materials, the proteins alone contribute nitrogen, sulphur, and phosphorus, although these elements are also present, as we shall see later, among the mineral constituents of foods. Nitrogen is generally considered to be, *par excellence*, the element necessary for living processes, since it is common to every protein; but sulphur, which occurs in nearly all, and phosphorus, which is present in some of the most characteristic and important (for man), are equally indispensable.

§41 Our chemical knowledge of the proteins is by no means as far advanced as is that of carbohydrates or fats; the great complexity of the proteins has more than offset the extra attention they have received on account of their supreme importance. We do, however, know something of the simpler compounds, called amino-acids, of which the complex intercombination forms proteins in the body. Since, moreover, the animal organism has very limited powers of making amino-acids for itself, it seems that all the amino-acids needed by man for the anabolism of his own tissue protein must be exogenous—that is, supplied from the outside in his food.

The complexity of the proteins is caused not only by the number of different amino-acids, but also by the ability of the same amino-acids to combine together in different ways. Over twenty amino-acids have been isolated from vegetable and animal sources, and fully characterized. Some of these, it is true, occur but rarely, or in very small quantities, or both; however, it is

highly probable that still others await discovery. If so, they are not likely to be at all abundant, but that does not mean that they may not be of an essential nature. The exact method of combination is known only for some of the very simplest compounds of amino-acids, and these are the peptones rather than proteins proper. This is perhaps made easier to appreciate if we remember that the following series of substances shows a *decreasing* order of complexity—Protoplasm  $\longrightarrow$  proteins (etc.)  $\longrightarrow$  metaproteins  $\longrightarrow$  proteoses  $\longrightarrow$  peptones  $\longrightarrow$  polypeptides  $\longrightarrow$  amino-acids  $\longrightarrow$  elements (carbon, hydrogen, nitrogen, oxygen, phosphorus, sulphur).

§ 42 Proteins, then, have complexities in common with both the carbohydrates and the fats, as well as some special complexities of their own.

The individual glycerides of a fat are limited in variety by the fact that there can be at most only three different fatty-acid molecules combined with each molecule of glycerol, though the great variety of the glycerides mixed together in any particular fat largely nullifies this handicap. The carbohydrates, on the other hand, are mostly complexes of a single sugar—glucose—but there seems hardly any limit to the number of glucose molecules that may be condensed together in one molecule of a “higher” carbohydrate.

The proteins, however, have it both ways. There are many different individual amino-acids; any two of them can combine together in at least two different ways; there appears to be no limit to the number or the variety of amino-acids that may be combined together in a single protein molecule. Moreover, different individual proteins may be associated together in a particular animal or vegetable tissue. Since the chemist gets information about the constitution of proteins by splitting them up into their constituent amino-acids, he is on the horns of a manipulative dilemma. If his methods of scission are too mild, he will have an intractable mixture of proteoses and peptones to handle; if the methods are rigorous enough to carry the splitting process right through to the amino-acid stage, he may get no



knowledge about the number of individual proteins contributing to his original mixture.

Various methods are used by the biochemist to separate individual proteins from each other without materially affecting their individual structures, but it must be conceded that his results often permit of legitimately different interpretations. No one even to-day can, to take a single instance, say with certainty how far the casein precipitated from milk by rennin (the ferment of rennet) differs from caseinogen, the phosphorus-containing protein present as such in liquid milk. Indeed, it seems certain that the very act of precipitation *must* effect some irreversible change, for no one has ever been able to get a rennet curd back into a solution identical with the original milk.

§ 43 What has already been said about protein chemistry, and the difficulties of protein chemists, should have prepared the reader for finding our information on the subject much less comprehensive than our knowledge of fat and carbohydrate structure. Indeed, from the point of view of nutrition and physiology, we can say that next to nothing is known about the proteins themselves. But our knowledge of amino-acid metabolism is much more considerable. Since, moreover, the proteins cannot pass through undamaged cell-walls, and it is only the amino-acids derived from the food proteins that ever get through the intestinal wall *into* the body, our ignorance is in practice not so serious as it might otherwise have been.

A considerable number of amino-acids are known to the biochemist—by which the reader must understand that they have not merely been split off from proteins by hydrolysis, but have been separated as pure chemical compounds and in most instances synthesized. This means that their precise structure is known in all details, as are the structures of monose sugars and fatty acids, even down to the right- or left-handed arrangement of the individual atoms in particular pairs of amino-acids that resemble, and differ from, each other much as do the two members of a pair of gloves.

The simile of gloves is at one and the same time too simple and too complicated. We can get a little closer to a rough picture of protein structure based on amino-acids if we imagine, first, that one glove of every pair has been lost, the left glove of some and the right of others. This corresponds with the fact that some of the amino-acids only occur naturally in the left-handed and others only in the right-handed form. Next, we shall find that the gloves differ not only in material, but also in the number of fingers. However, all the fingers except one, or at most two, on any glove are cut off and sewn up, leaving only a single finger, or two, and a thumb available. Any thumb can "combine" now with any finger. Obviously, two single gloves can link up in two differing ways; if we represent thumbs by capital letters and fingers by small letters, Aa and Bb can combine either as *Aa-Bb* or as *Bb-Aa*. Each of these can, in turn, link up with another glove of the same kind or of a new kind. So, for example, we might have *Bb-Aa-Aa*, or *Bb-Aa-Bb*, or *Bb-Aa-Cc*. It is obvious that the more gloves—amino-acid molecules—are linked together, the larger the number and the more varied the nature of the resulting compounds, which are the *polypeptides* already mentioned (p. 37, § 41, par. 2), passing with increasing complexity through the peptones up to the proteins themselves. At this level of complexity the possible number of protein structures is, indeed, not merely astronomical, but practically infinite.

Fortunately the number of these structures really occurring in nature is finite—as far as we know! Reference has already been made to the difficulties involved in characterizing individual proteins, but it seems likely that most of the important ones, and particularly all the most important ones in foods, have been characterized and separated in a more or less pure and uncontaminated state. It would be pointless to weary the reader by recounting all their names; it is perhaps sufficient to remind him of egg-albumin, gliadin (one of the proteins of wheat), casein (the characteristic protein of the milk of all mammals), and myosin (one of the proteins of muscle—that is, of meat). Gelatin is sometimes inaccurately

referred to as a protein: strictly speaking it is a man-made break-down product of an important body protein, collagen, which is a kind of living mortar between the structural bricks of the animal body. However, gelatin retains many of the characteristic physical and chemical properties of a true protein.

§44 There is one feature of the amino-acids that is worth noting, partly because of its inherent interest and partly because it is common to many other naturally occurring substances. As has already been mentioned, pairs of many amino-acids are known of which the two members are identical except that in structure one is the "mirror-image" of the other. The two gloves, so to speak, are made of absolutely identical material, and have the same number of fingers and thumbs. Of the two gloves only one occurs in nature, but the opposite member has in most instances been made by the chemist. The two members of such pairs are known to the chemist as "optical isomerides," which means, in less concise language, that their solutions behave to certain forms of light in opposite manners. One form is known as the *dextro*- (right-handed) isomeride, the other as the *lævo*- (left-handed) isomeride.

It is a well-known fact of biochemistry that the two members of such pairs may have different physiological action; one member, indeed, is sometimes completely devoid of the action characteristic of the other. More often the difference is merely quantitative. Thus the effect of natural adrenalin—the *lævo* form—in contracting the capillaries when it circulates in the blood-stream is several times that of the *dextro* form. (Both forms have been made synthetically, and the active *lævo* form may to-day very often be "fine chemical" product, and not the material extracted from mammalian adrenal glands.)

When animals are fed on "synthetic" mixtures of amino-acids, in place of the proteins of which they constitute the units, it is found that sometimes only one isomeride—the naturally occurring one—can be utilized, while in other instances either form seems to be acceptable. This varying ability of the animal organism is a departure

from the otherwise almost universal rule that one member of a pair of optical isomerides is much less valuable than the other or even of no value at all.

§45 The characterization of the chief proteins involves three kinds of information. The first, the qualitative, involves finding out *which* amino-acids are present, and this has been done pretty completely; the second, the quantitative, requires the establishment of the relative amounts of the individual amino-acids, and this is sometimes accurately known. But the third, the structural, necessitates a knowledge of how the different amino-acid molecules are linked together—which fingers to which thumbs—and of this very little indeed is known. There again, however, the lack of knowledge is more distressing to the organic chemist than to the nutritionist or dietitian, for it is the amino-acids themselves that circulate in the organism, to be built up into the characteristic proteins of that organism or, to a lesser extent, to be burnt up as fuel in a manner similar to fat and carbohydrate; when the latter occurs, the carbon and hydrogen of the proteins leave the body as carbon dioxide and water along with the combustion products of fat and carbohydrate, but the nitrogen has to be lost in the excreta, the hair, the nails, the perspiration and, above all, the urine. In this it is found chiefly as the fairly simple substance urea—a compound of great importance, in spite of its simplicity; for it is a highly typical animal product, yet it can be synthesized by a series of straightforward reactions from even simpler substances classified as entirely inorganic—ammonia gas and carbon dioxide, for example. This is one reason why the chemist regards the distinction between inorganic and organic compounds as merely one of convenience in classification and not as implying any mystical qualitative difference between them.

§46 For the reasons already given, we can turn from the chemistry of the proteins as such and consider a little further some features of the amino-acids. Of these, two are perhaps the most important. First is the fact that they themselves are of architectural use to the eater

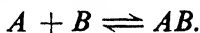
of proteins only in so far as they can furnish an essential part of his own body-protein, whether directly or by being changed into some other essential amino-acid: secondly, the fact that breakdown of superfluous or unusable protein normally involves the excretion of urea through the kidney, so that a protein consumption largely in excess of optimum requirements may involve putting an extra strain on this vital organ. It must be admitted, however, that proof of a high protein diet's having an adverse effect on the normal kidney does not seem available. Anyhow the normal kidney can take care of a very considerable protein surplus, but the diseased kidney may not be able to do so. One of the easiest ways of discovering impaired kidney function is by the simple tests for protein in the urine; where the proteinuria or albuminuria, as it is called, is really high, the impaired kidney function of which it is the sign may be so serious as to prove fatal.

## CHAPTER IX

### AMINO-ACIDS IN THE BODY

§ 47 FOOD proteins, as we have seen, must be catabolized—broken down—in the body before they can be anabolized—built up—to form the natural proteins of that body. There must, therefore, be present mechanisms for carrying out both processes, as well as for getting rid of any amino-acids not wanted at all or wanted only in smaller amounts than the food has supplied. Here, as with the catabolism of fats, discussed in Chapter VI, the enzymes have a task; the particular ferments involved are known as proteolytic or proteoclastic enzymes. Different ones control the various stages of breakdown from complex protein to amino-acids (as outlined on p. 37).

Enzymes also take part in anabolism; they have the power, common to many catalysts, of being able to work both ways, as it were. The action of a catalyst always tends to an equilibrium, which in its simplest form the chemist expresses symbolically as



§ 48 The extent to which the chemical change goes in one direction or the other depends on numerous factor, but mainly on the rate at which the reaction products are removed from the influence of the catalyst. For example, if the reaction  $A$  with  $B$  is taking place in a solvent, and if the compound  $AB$  is insoluble in that solvent, the reaction will continue until all  $A$  and  $B$  have combined to form  $AB$  and have consequently been precipitated from the solution. On the other hand, if  $AB$  is a solid and is being converted to two gases  $A$  and  $B$ , and if these are dissipated in one way or another, all of  $AB$  will in due course be broken down to  $A$  and  $B$ .

In the living tissue, naturally, a far more complex state of affairs exists; nevertheless the *biochemical* reactions taking place therein are of a "balanced" type funda-

mentally of the same kind as the above examples. The proteolytic enzymes of the stomach, pancreas and intestine are able to effect a fairly complete breakdown of food proteins to amino-acids so that these, being soluble in the juices of the alimentary tract, get carried away from the site of the enzyme action and pass through the walls of the tract into blood-stream and lacteals; the enzymes thus have no amino-acids available for anabolism—rebuilding to peptones, proteoses and so on—and proceed to catabolize more protein.

§ 49 If the reader deduces from the last sentence that the *same* enzymes take part in protein catabolism and in protein anabolism, he will have deduced right. There is experimental evidence that they are indeed capable of catalysing both processes. When the amino-acids in the blood-stream reach the tissues in which protein has to be built up—notably the muscles, but indeed also every cell in the living body—enzymes essentially similar to the digestive enzymes almost certainly play the main part in the re-synthesis. It seems likely that many different enzymes must be involved in protein anabolism; certainly the cellular conditions under which they perform their functions must be extremely diverse.

Owing to the difficulty, already discussed (see p. 37), of accurately characterizing individual proteins, it is quite impossible to say even approximately how many separate proteins occur in the human body. They are, however, certainly numerous, if one may argue from the proteins of other animals. It can easily be understood that exact knowledge of even the amino-acids present in the proteins of live human tissues is almost impossible of achievement by direct analysis, and this makes it difficult to know what amino-acids are essential to man and in what amounts. Hence we are dependent to a considerable extent on animal experiments and argument by analogy.

§ 50 Investigations into the amino-acid requirements of experimental animals, and also of farm animals, have been carried out in dozens of laboratories for several decades. A diet of precisely known amino-acid content, lacking one single amino-acid, but containing all other

recognized dietary essentials, can be prepared and its effect on a group of animals compared with that of the same diet to which the missing amino-acid has been added. The increases in size and weight, the general health, the fertility and other characteristics of the two groups of animals may all furnish evidence by which to decide whether or not the particular animals can dispense with the particular amino-acid. Analysis of the animals' body-proteins will then often make it possible to know if the animal has only appeared to do without the amino-acid, or if it has been able to synthesize it from some of the other food constituents. There is a considerable amount of published information on this subject; some of the earlier work needs rather critical evaluation, because it was carried out before present-day knowledge of vitamins and other "accessory" substances was available. The beneficial effect of a particular amino-acid might have been due to its contamination with some trace of an unknown essential food constituent, absent from all the other dietary ingredients. Nevertheless, despite some difficulties in interpreting these investigations, the general picture is fairly clear.

In a series of recently published papers, two groups of American scientists, Holt and Rose and their respective colleagues, have reported a new attack on the problem of human amino-acid requirements. Certain pure amino-acids—some laboriously separated from the laboratory breakdown products of proteins, others synthesized by organic chemists—having become available in sufficient quantities for experiments on human beings (albeit sometimes at a price not to be contemplated by most British investigators!), the American teams got certain volunteers to submit themselves for days or even weeks at a time to diets in which practically the whole of the utilizable nitrogen was present as a mixture of these amino-acids.

It was found that the removal from the synthetic diets of certain individual amino-acids caused the human experimental subject to go rapidly into negative nitrogen balance: the balance quickly became positive when the



missing amino-acid was restored to the diet. From this work, and from certain independent information obtained on other occasions, it has for the first time become possible to write down for man, as well as for rat, mouse, dog and chicken, the names of all the amino-acids found to be essential by the above-mentioned criterion. In so far, moreover, as the diets used were indeed free from all protein and amino-acids other than those intentionally introduced, and in so far as these were free from significant quantities of any other contaminating amino-acids, it seems quite likely that the list is correct both inclusively and exclusively, though of course it is—and probably always will be—impossible to say whether the same selection of amino-acids (in the same approximate proportions) are also adequate for growth and reproduction in man. As a matter of interest, the established facts are summarized in Table I, p. 47.

From the work outlined above, it appears that nearly half of the naturally occurring amino-acids are essential to man (and other animals, including birds); they must, that is to say, be supplied in the animal's diet, in either vegetable or animal food. Ultimately, therefore, the animal kingdom depends on the plant world for its supplies of protein, as for its carbohydrates and minerals. It is true that excess protein can be rather extravagantly transformed, *via* its amino-acids, to carbohydrate; but very few wild animals, and still fewer human beings, find it desirable or necessary to adopt so uneconomical a regime.

§51 Plant proteins differ from animal proteins both in the number and in the proportions of their constituent amino-acids. It is because of this that the physiologist has come to speak of some proteins as "good" in the biological sense and of others as "poor." (The antithesis is curious and should not be indiscriminately applied to the study of social phenomena generally.) A "poor" protein is one that is unable by itself adequately to support animal life; a "good" protein can do so. Few proteins are perfectly "good," but, generally speaking, animal proteins are "good" and vegetable proteins are "poor." Even so, most single

TABLE I

*Amino-Acids Necessary for Man and Certain Other Animal Species*

	Growing Mouse	Growing Rat	Adult Rat.	Growing Chick	Adult Dog	Adult Man
Valine . .	?	+	+	?	+	+
Leucine . .	?	+	+	?	+	+
Isoleucine . .	?	+	+	?	+	+
Threonine . .	?	+	+	?	+	+
Lysine . .	+	+	+	?	+	+
Arginine . .	+	+	—	+	—	+
Methionine . .	?	+	+	+	+	+
Cystine . .	?	+	+	+	+	—
Phenylalanine . .	?	+	+	?	+	+
Tryptophan . .	+	+	+	+	+	+
Histidine . .	+	+	+	+	+	+
Glycine . .	?	—	—	+	—	?

+ = Essential.

— = Not essential.

? = Information not available.

animal proteins give inferior results to mixtures, and this is still truer of vegetable proteins. The reason should be fairly clear; we will take a simple numerical example. Supposing that the total body proteins of man, or some other animal, are represented by amino-acids *A*, *B* and *C* in the proportions

$$6A + 3B + 1C.$$

It is clear that a protein containing only one or only two of these amino-acids will not be able to support that animal's life, assuming that none of the amino-acids can be synthesized by the animal. If, however, it eats a

protein containing equal parts of *A*, *B*, and *C*, then it will have to get rid of three parts of *B* and five parts of *C* for every six parts of *A* that it consumes. The protein in question, though "good," would be extravagant.

If this protein can now be supplemented with a protein, "poor" in itself, that contains five parts of *A* to two parts of *B*, but no *C* at all, then the diet will be adequate if it contains quantities of the two proteins represented by

$$\begin{array}{rcl}
 & 1A + 1B + 1C & \text{six times} \\
 \text{plus } & \underline{5A + 2B} & \underline{1A + 1B + 1C} \\
 & 6A + 3B + 1C \text{ instead of } & 6A + 6B + 6C
 \end{array}$$

Thus there will have been a saving of *five* parts of the "good" protein, for *one* part of it will have been adequately supplemented by, say, two to three parts of the second "poor" protein. This *supplementary action* of proteins is of the greatest importance in nutrition. The foods containing the best proteins are the most expensive foods; they are also not very economical, in the physiological sense, as sources of energy. If, however, they can be taken as sources of the amino-acids absent from the "poor" proteins of many vegetable foods—notably cereals—then the "poor" proteins fulfil an invaluable function in supplying the amino-acids common to "good" and "poor" proteins alike.

§ 52 Consideration of the chemistry and physiology of the proteins in diet leads to the inevitable conclusion that a mixed diet is the best diet. The proteins of meat, milk and eggs, with those of cereals and legumes, taken together are biologically more useful—and certainly cheaper—than either group taken alone or in partial association.

Large sections of the population, especially among the simpler races, would find it a complete impossibility to obtain enough of the essential amino-acids from "good" proteins alone, even if the diet implied by such dietary restriction were not undesirable for other reasons. For those people, by economic, climatic or geographical circumstances, as for all mankind by physiological necessity,

variety is not merely the spice of life, but the soundest road to amino-acid "salvation."

The old identification of "good" with animal protein and of "poor" with vegetable protein is now recognized to be only a very rough approximation. Gelatin, though not, as we have already seen, strictly a complete protein, is certainly of very low nutritional value: on the other hand, the proteins of yeast, a typical unicellular plant, and those of green leaves have very nearly, if not quite, the biological value of meat and milk protein, though the *amount* of protein conveniently to be obtained from the second source is relatively small.

§ 53 There is one other feature of protein metabolism that needs a brief account here. We have already seen that the calorific equivalents of the principal food constituents are accurately known from purely physico-chemical experiments. The amount of heat that should be derived from them in the body can be calculated to a nicety. If a man in a state of rest takes a certain amount of food, in which the proportions of fat, protein and carbohydrates have been determined by analysis, then his body should be able to produce the corresponding amount of heat and no more. By what the physiologists call animal calorimetry, the true heat production during rest—called the basal metabolic rate—can also be measured with considerable accuracy.

It is invariably found that this amount of heat is in fact more than can be accounted for by the amount of food consumed on the assumption that it has all been completely burned up in the body. Further, this excess heat production is almost entirely due to the protein in the food, and indeed to certain, but not all, of the amino-acids therein, and hardly at all to the fat or carbohydrate. This effect of protein on the production of bodily heat (or energy of movement, if the subject is not at rest) is called the "specific dynamic action" of the protein or amino-acid.

§ 54 The exact mechanism by which amino-acids exert this specific dynamic action is not fully understood. Possibly it is associated with the conversion of the amino-

acids to carbohydrate. Be that as it may, one thing is clear: the heat must come from somewhere. It does not come from the food and must therefore be derived from the combustion of cell constituents already present in the body before that food was consumed. This stimulating effect of food-protein on bodily oxidations has an important consequence. It indicates clearly that a high-protein diet should be efficacious in enabling the body to burn up its existing stores of fat and carbohydrate. A diet of lean meat and lean fish must therefore be a "slimming diet," while hardly at all depriving the body of essential food constituents, for we have seen that protein can be converted into carbohydrate and then into fat, if required. But in being so converted it will cause the combustion of more fat or carbohydrate or protein than it can itself replace. The use of high protein diets, when not otherwise contra-indicated (by impaired kidney action, for example), as a means of reducing excessive weight is, in fact, advocated and does, in fact, succeed.

## SECTION III—MINERALS

### CHAPTER X

#### THE MAJOR MINERAL ELEMENTS

§ 1 IN this chapter I am using the word “mineral” in a wide sense; it is taken here to include all the elements, non-metallic as well as metallic, that occur in foods, apart from the four appearing in organic combination as fats, carbohydrate, and protein—that is, excluding carbon, hydrogen, oxygen, and nitrogen, but *including* sulphur and phosphorus, although these do occur organically combined in many proteins. The “mineral” elements, in short, are roughly those that the chemist reckons as occurring in the “ash” of a food after ignition.

§ 2 Just as the constituents of food as a whole can be roughly divided into “major” and “minor”—some ninety-five parts by weight out of a hundred being accounted for by fats, carbohydrate, protein, and water—so can the odd 5 per cent. be roughly divided into those elements and compounds that preponderate and those that, however important, are required only in “traces.” These trace substances include the vitamins and possibly some other complex organic compounds of less well-defined properties and composition, as well as certain individual elements—both metallic and non-metallic; the preponderating elements, on the other hand, are few in number, and the recognition of their importance belongs to the period of “classical” nutritional theories. Even those who did not in the least suspect the dietary insufficiency of foods containing only fat, carbohydrate, protein and water, would have agreed that calcium was needed, to form teeth and bone, and iron, to form blood, and that certain other elements besides carbon, hydrogen, nitrogen, sulphur and phosphorus were probably not present in the human frame as accidental contaminants.

§ 3 On the other hand, it is even to-day by no means certain, however likely, that traces of copper, manganese, aluminium, zinc, cobalt and fluorine have an essential part to play in the metabolism of human beings—whatever may be true of experiments with laboratory animals. Intermediate between these and the “major” mineral elements are others, in particular sodium, potassium and magnesium: present in intermediate amounts, they are both ubiquitous and indispensable. Iodine has a unique position in that it alone of the trace elements is known for certain to be essential.

§ 4 The division of these essential chemical elements into two main groups, though it may at first sight appear a little arbitrary, is in fact historically justifiable as well as a convenient method of presentation. In this chapter, therefore, we shall consider briefly all the *major* items in this group of *minor* food constituents, both those recognized in “classical” nutrition and those of which the essential nature has been more recently established. In the next chapters we shall briefly discuss the “trace” elements and so, we hope by an easy transition, pass to the vitamins, which, though of highly complex organic structure, do their jobs in quantities of the same order as the “trace” elements themselves. To take a single example—the amount of copper required daily by the young growing albino rat is about 8 micrograms per day; the amount of aneurine chloride (vitamin B<sub>1</sub>) required daily by the same animal is about 4 micrograms (a microgram is one sixty-thousandth of a grain, which is itself equal to 60 milligrams, the microgram being exactly one-thousandth of a milligram). The discovery of the vitamins, their natures and functions, has occurred during the same period as that of the trace metals; both discoveries were undoubtedly to a large extent made possible by contemporaneous improvements in laboratory methods, especially in the technique of animal experimentation.

§ 5 Bone and teeth consist largely of the carbonate and phosphate of lime (calcium oxide). The carbon, oxygen and phosphorus of these salts could, of course, at any rate in theory, be derived from the major food

constituents, since oxygen occurs in all of them, carbon in fats, protein and carbohydrate, and phosphorus in many proteins, as well as in the phospho-lipids, closely associated with both animal and vegetable fats, although never present as more than a fractional percentage by weight in food. (A fairly well-known phospho-lipid is lecithin, present in egg-yolk, in soya-bean oil and in other natural fats and oils.) Even, however, if the phosphorus of protein and phospho-lipid were adequate for the formation and maintenance of bones and teeth, there would still be other phosphorus requirements of the body to meet and especially, as the perspicacious reader will have foreseen, the requirements for building up the body's phospho-proteins and phospho-lipids.

§ 6 The phosphorus required for formation of bones and teeth occurs in foods partly along with calcium, combined as one or more of the various simple or complex phosphates of calcium. Neither element is of any use for skeletal purposes without the other. It is, however, hardly to be put down to luck that man, like other vertebrates, finds together in foods two elements needed by him for the same bodily purpose. It is more logical to argue that the occurrence together of these two elements in plants and lower animals was itself a determining factor in allowing the evolution of organisms that require both these elements together for an essential part of their structure.

Whatever the evolutionary truth of this matter, the fact is beyond dispute. Man must have both elements for his bones and teeth: moreover, to direct this exogenous calcium and phosphorus to their structural end in bone and teeth, vitamin D is indispensable. Of this we shall have more to say later. Calcium and phosphorus also both circulate continuously in the blood-stream of adults, as of growing infants and children. The amounts of these two elements present in the blood of healthy individuals oscillate only between rather narrow limits; small departures from these lead immediately to pathological symptoms; large departures may have fatal results. The metabolism of calcium and phos-



phorus is at least as important to the living vertebrate organism as that of any other element, not even excepting iron, vital for blood formation; fortunately both elements are widespread (though calcium is comparatively abundant only in milk and cheese and a few vegetable products) and are generally absorbed and assimilated with great ease. Phosphorus is extensively distributed in both plant and animal tissues.

§ 7 Magnesium, an element related to calcium, seems, on the evidence of animal experiments, to be essential to normal health; its metabolism is closely connected with that of calcium and phosphorus, and it is mainly distributed along with them in foods. It seems particularly concerned with the physiology of the nervous system and the capillary blood-vessels.

There seems no reason—at present—to believe that the other elements closely related to calcium—strontium and barium—or the elements related to phosphorus—arsenic and antimony—have any effect other than a toxic one on the human organism, and they may therefore be allowed to pass from the picture being attempted in this book.

§ 8 Iron, though it has other parts to play in the complex chemistry of living processes, is of primary importance to man and other vertebrates because it is an essential element in hæmoglobin, the red pigment of the blood-cells; it is also present combined in enzymes that control the respiration of individual cells—the burning up of cellular contents to give heat and energy. Hæmoglobin has the fundamental task of carrying oxygen from the lungs to the various parts of the body where the fuel constituents of food are burned. When the iron supply is insufficient, owing to shortage in the food or to defective absorption or inefficient utilization, anæmia develops—one of the commonest of all “deficiency diseases” afflicting the human races. This type of anæmia may be aggravated, if not caused, also by shortage of protein in the diet. This is no place to discuss the different types of anæmia, but it is necessary to mention that those due to hæmoglobin shortage have certain well-marked characteristics that distinguish them clearly from, for example,

pernicious anæmia, which is due to quite a different cause and is in no way to be cured, or even mitigated, by treatment with iron.

§ 9 Views as to the best dietary sources of iron have swung a rather pendulum-like course during recent decades. The determination of the amount of assimilable iron by tests on animals is extremely laborious and the results are not very accurate, nor do they necessarily apply to man. Because of the ease with which the body stores iron—mentioned below—and our meagre knowledge as to where and in what form it is stored, the method of the balance experiment, so valuable in establishing nitrogen requirements, cannot be used. Certain facts, however, seem to emerge as fairly well proven, out of a large body of experimental data, laboratory and clinical, some of which are difficult to interpret and some even inconsistent with others.

Iron in the mammalian body is present as several organic compounds, of which the best known is hæmoglobin; the amount of hæmoglobin in the blood, and of the red cells containing it, can show with considerable accuracy the absence or presence of anæmia and the extent of its remission under treatment. That is probably why hæmoglobin was formerly recommended as a means of treating iron deficiency. Modern researches have shown this view to be unjustified; the prescription of iron in “natural” association with chlorophyll, the green pigment of plants (itself free from iron), is based upon a like error. Even some of the less complicated iron compounds, such as the “scale salts” official in many pharmacopœias, are inferior remedies. For the subject suffering from nutritional anæmia, or even from some of the pathological forms, the simplest iron salts have much the greatest value.

When we pass from medicinal treatment with iron-containing drugs to consider what foods are the best sources of iron for *preventing* nutritional deficiency, the position is much less clear. It is, indeed, no longer held that raw red meat is the best, or even an especially useful, source of iron—that is to say that its iron, weight for

weight, is better absorbed or utilized than the iron in other types of food. It seems clear, too, that cereals and green leaves are among the best food sources of "available" iron. The view that cooked meat is little, if any, better than raw meat for satisfying man's iron requirements, which was held by several distinguished investigators until fairly recently, seems now difficult to maintain in the face of further evidence. It appears to be broadly speaking true to say that the iron in cooked foods of all kinds, whether animal or vegetable, is much better assimilated than that of hæmoglobin or the more complex iron compounds of the pharmacy, and probably nearly as well utilized as that of the simpler salts, like the sulphate and chloride.

§ 10 In order to cure certain pathological anæmias and also—though to a less extent—simple nutritional anæmia, especially in the severer cases, very large doses may be required even of the best (that is, the simplest) iron compounds. Some investigators have suggested that this may be explained in terms of the amount present of some "trace" element, accompanying the iron in small and variable quantities. We shall return to this question of "trace" elements in the next chapter.

More recently experiments on normal subjects and anæmic patients have pointed to a rather different explanation. Apparently a deficiency in hæmoglobin, even when of nutritional origin, cannot always be made good unless much larger quantities of iron than are required to make the hæmoglobin level normal are consumed and actually absorbed. It is known that by giving extremely large doses of iron, of which a great deal is almost immediately lost in the fæces, a store of iron can be built up in the body, very little passing out by way of the kidneys. It may therefore be necessary in some persons to build up, as it were, a considerable "pressure head" of iron in the body in order to promote adequate hæmoglobin formation. It is worth remarking here, by the way, that high doses of iron compounds are, despite popular and even some medical opinion, not in the least constipating, though small doses may be!

§ 11 Whatever the explanation of this mysterious storage of iron at some unknown site or sites in the body, it has one obvious corollary for the dietitian. Excess of iron in the diet is harmless; deficiency of iron leads to nutritional anæmia that may require prolonged treatment with large doses of iron to restore a normal blood picture. There are in this country many anæmic children and adolescents. The advocates of increased milk consumption—essential though this is for public health on other grounds—are doing nothing to remedy this; milk, as we shall have occasion to point out later, is a very poor source of iron. For this we have to depend on green vegetables, some cereals—especially high extraction flour and oatmeal—legumes, liver, meat and egg-yolk. The iron in wheat is distributed throughout the grain, though it is richest in the outside branny coats. This is to some extent set off by the fact that these coats contain a substance called phytic acid, which forms, with iron (and incidentally also with calcium), salts—phytates—so insoluble that they may yield none of their metal to the fluid intestinal contents and so fail to contribute to the body's requirements. The embryo, on the other hand, is rich in iron, but contains little phytic acid. For purposes of preventing nutritional anæmia, a high extraction flour, with a minimum of true bran, has much to commend it, and "national flour" from 1943 onwards has been most satisfactory in this respect. There is, moreover, some evidence to suggest that in the fermentation of dough and the baking of bread some destruction of phytic acid occurs, with a consequent reduction in its immobilizing effect on iron and calcium in the bread and other parts of the diet.

§ 12 It is necessary to point out further that women almost certainly need more iron than men. Hæmoglobin determinations on similar groups of men and women apparently quite free from outward manifestations of anæmia almost always show a slightly lower figure for the women. Moreover, that figure can be raised to the male level by a moderate iron dosage that has no effect on the hæmoglobin in normal male blood.

This gives strong presumption that the women are, indeed, slightly anæmic—as opposed to the suggestion that women's “physiological level” of hæmoglobin is lower than man's. Apart from the requirements of the fœtus in pregnancy (and also the loss of iron in blood at parturition), the monthly loss of blood during menstruation is probably an important factor in determining the higher iron requirements of the adult woman.

§ 13 The commonest salt known to man is, not unnaturally, common salt. Indeed, its presence in sea-water has determined the whole course of evolution. Those readers who have a taste for both biochemistry and philosophical speculation will find this subject expounded in a most fascinating manner in Dr. Ernest Baldwin's *Comparative Biochemistry* (Cambridge University Press, 1937: 5s.). Common salt—sodium chloride—is as widely distributed inside man as outside: every body-fluid, and especially the blood-stream, contains more sodium than any single other metal. Chlorine is present to a somewhat greater extent and is the most abundant non-metal of the “minor” constituents in the human frame.

A metal closely related to sodium is potassium, and this is present mainly in the blood corpuscles rather than in the serum in which they circulate: incidentally the corpuscles contain no sodium at all.

§ 14 Substances in solution in an enclosed space exercise a pressure on the enclosing walls: this is called “osmotic pressure.” If the walls are permeable, and if there is a watery fluid on either side of these walls, water will pass from one side to the other until the osmotic pressure is the same on both sides. A strong solution inside such a permeable container will become weaker by infiltration of water if there is a weak solution of salt on the outside, while the outside solution will thereby become more concentrated. Such osmotic phenomena are to a large extent independent of the chemical nature of the substances in solution: those substances may therefore be regarded as of physical rather than chemical importance. The main function of sodium, combined

with chlorine or carbonic acid as chloride or bicarbonate, is of this physical nature, whereas potassium acts in a more specifically chemical manner. A shortage of sodium in the food of experimental rats permits normal growth for a relatively long period, but potassium deficiency results in early death.

§15 Sodium, potassium and chlorine are not only abundantly present in food, but they are also very widely distributed. Almost any diet is probably an adequate source of all three; the biological origin of taking salt with meals is rather difficult to explain. The requirements for these elements are, moreover, not great in normal circumstances, but it should be noted that perspiration is very rich in sodium chloride. Consequently continuous very violent exercise or hard manual labour may result in a temporary shortage of salt in the body-fluids, and the condition known as miner's cramp is due entirely to this. The miner's remedy—to take extra salt and even to add salt to his drinks or to any water he may consume—is the result of traditional knowledge that is physiologically unimpeachable. There is, however, some evidence that gradual acclimatization to work at high temperatures may make an increase of salt intake unnecessary; the body, apparently, learns to sweat more water and less salt and so not to disturb the balance of osmotic forces between the fluids inside and outside its cells.

§16 Chlorine plays one other important part, besides keeping sodium and potassium, as chlorides, dissolved in body-fluids and tissues. It is needed to form the hydrochloric acid—a compound of hydrogen and chlorine—secreted by the stomach, where it takes part in some of the early digestive processes, particularly in assisting the breakdown of proteins, as we have already described. The actual *amount* of chlorine required for this purpose is, however, relatively small. The quantity of hydrochloric acid in gastric juice seldom reaches more than one quarter per cent. In other words, a pint of gastric juice contains an amount of hydrochloric acid that could be derived from  $2\frac{1}{2}$  grams of common salt—under

one-tenth of an ounce. In fact, of course, the volume of gastric juice in the stomach is much less than one pint: the salt equivalent of the hydrochloric acid in the normal stomach, even when it is at its maximum after a meal, does not exceed half a gram. As the equivalent of 10 grams of sodium chloride may be lost daily in the urine, apart from any passing out in the fæces, it is clear that the amount of chlorine needed for the formation of gastric hydrochloric acid, essential though it is, is small compared with other bodily requirements. The blood alone, in a man weighing 10 stone, contains chlorine equivalent to about 60 grams (2 ounces) of sodium chloride or 80 grams of potassium chloride.

## CHAPTER XI

### THE HORMONES—IODINE

§ 17 A BRIEF consideration of the endocrine or ductless glands—also called the glands of internal secretion—is necessary at this point. The elements from which the active principles of these glands, known as hormones, are built up must originally have come from food. There is no evidence whatever to suggest that man ingests any of his hormones as such, and it may certainly be accepted that he can normally synthesize all of them.

The glands of the animal body are divided broadly into two classes: those with special channels, called ducts, in which their secretions are carried into the appropriate organs, and those that discharge their secretions directly into the blood-stream. There is no reason why one gland should not fall into both categories; the pancreas, in fact, does.

§ 18 During recent years much progress has been made in identifying, isolating and even synthesizing the individual hormones. One of them, adrenaline, has been available as a synthetic “fine chemical” for more than twenty years. Adrenaline is the hormone from two small glands situated quite close to the kidneys; they are sometimes called the suprarenal glands, and the hormone has been called epinephrin and suprarenin. The terms adrenal and adrenaline, however, are most generally accepted. Its property of raising the blood-pressure is due to its ability to constrict the smaller arteries or arterioles—a fact made use of by the dentist, who frequently injects local anæsthetic and adrenaline simultaneously.

§ 19 More recently thyroxine, the essential substance from the thyroid gland, has also been synthesized. It is distinguished from all other known hormones by containing iodine, and it is for the production of thyroxine that iodine is required as an essential nutritional element.



The thyroid gland plays a fundamental part in the control of the body's oxidative processes. The amount of energy required by a resting organism—for maintenance of body temperature, for the action of the muscles that continue to work even during sleep, such as the heart muscles—is called the basal metabolism. Lowered thyroid activity reduces the basal metabolic rate and has many other serious effects. In extreme cases it may lead to a condition known as myxœdema, or to cretinism, or to one form of goitre. Investigations in many parts of the world have shown a close correlation between the incidence of endemic goitre and the amount of iodine in the soil, food and drinking-water. Exophthalmic goitre, however, is due to excess of thyroid activity—hyperthyroidism; it is not due to iodine shortage or cured by its administration.

Goitre is never endemic near the sea or in other places where iodine is relatively abundant; where, however, endemic goitre exists, iodine supplies are always found to be abnormally low. This is particularly liable to occur in regions cut off from the sea by mountain ranges. It is possible practically to exterminate endemic goitre by enforcing legally the iodination of table-salt and cooking-salt. The amount needed is relatively very small; the requirement of the average adult is about one and a quarter of a grain *per year*! Quantities of iodine far in excess of this amount are quite harmless—it is doubtful if they would seriously affect the few rare individuals suffering from hyperthyroidism—that is, excessive production of thyroxine; the cost of adding to all salt for human consumption an amount of sodium iodide sufficient to ensure that everyone obtained, say, an additional grain of iodine spread over twelve months would be almost negligible. (Children appear to require about three times as much iodine as adults.) There is, therefore, a good deal to be said for compulsory iodination of salt in all countries, even where there is no question of endemic goitre.

However that may be, there remains the indubitable fact that certain forms of hypothyroidism—an *endocrine*

disturbance—are undoubtedly caused by *wrong food* and can be cured by making good the dietary deficiency. This is the only clear and proven instance of such a connection.

§ 20 It must, of course, not be forgotten that, as has already been pointed out, *all* our hormones are ultimately of dietary origin. But the fats, carbohydrates, and proteins of our food contain all the elements necessary for building up other hormones of which the elementary constitution is known. Some contain only carbon, hydrogen, and oxygen, like the fats and carbohydrates. Others contain nitrogen as well, and must presumably derive this from dietary or bodily proteins (see Table II, p. 65). At least one hormone—insulin—contains sulphur, and this may be derived either from certain proteins (or their appropriate amino-acids) or, less likely, from mineral sulphates.

It may be objected that the new-born infant's body contains sufficient of all these elements to cope quite easily with all its hormone requirements to the end of its days, and that therefore no external origin of the hormonal elements need be postulated. To this the answer is simple: the life of the organism does not begin at birth, but at conception: the fertilized ovum—at any rate in mammals—is clearly not sufficiently endowed for its subsequent endocrine demands. If the new-born infant were so endowed, this could only be the result of intra-uterine nutrition. In other words, if the elements required for our hormone production are not derived from our food after our birth, they *must* be derived from our mother's food before our birth, for the embryo certainly does not carry with it into the world a full adult complement of hormone. Of course there is no need to assume so remote a connection between food intake and hormone production: the simpler hypothesis is more likely the right one. Our hormones, like all other bodily secretions, are built up from elements ingested in our food. Moreover, it is well in this connection to recall what was pointed out earlier (p. 12) about the dynamics of bodily structure: what is

true of the body's stores of protein and fat is doubtless also true of the hormones—namely, that they are continually being broken down and re-formed from fresh supplies of the essential elements which must ultimately come from the food. This has, indeed, been experimentally verified for the thyroxine iodine of the human thyroid gland.

§ 21 When we remember the relationship between iodine intake and thyroid secretion, it is not at all fantastic to suggest that there may be other disorders of a similar nutritional origin caused by a lack of some element or group of elements necessary for the production of a hormone—either by actually entering into its composition or by taking part in the chemical reactions necessary to produce it.

Of the hormones besides adrenaline, insulin and thyroxine, little can be said here, since nothing but a general connection can be traced between them and nutrition. One hormone, only recently isolated, is that of the adrenal cortex (the rind of the gland); it is called corticosterone and has been successfully used in the treatment of Addison's disease, hitherto always fatal in a very short time. The disease is characterized by bronzing of the skin, nervous symptoms, gastro-intestinal disturbance and sexual decline. A clue to its cause was given by the fact that it showed some marked similarities to the condition of rats deprived of their adrenal glands.

Unfortunately the pure hormone—whether it be of natural or of synthetic origin—is extremely expensive, and the necessary dosage is prohibitive to all but the very wealthy. It is therefore interesting to note—and germane to a discussion on connections between nutrition and endocrine physiology—that the dosage of corticosterone required for treatment of Addison's disease can be considerably reduced by increasing the amount of sodium chloride (common salt) in the patient's diet. The adrenal cortex, through its hormone, is apparently concerned in some very fundamental way with those osmotic phenomena mentioned in § 14 of the last chapter; for Addison's disease is accompanied by increased excre-

tion of water and of sodium chloride and a reduction of mineral matter in the blood. The successful combination of dietary with hormone treatment of this disease again calls our attention to the importance of research in the borderland between nutrition and endocrinology.

TABLE II  
*Elements and Hormones*

GLAND	ACTIVE PRINCIPLES	ELEMENTS PRESENT
PITUITARY ANTERIOR LOBE	Possibly several	Probably Carbon, Hydrogen, Nitrogen, Oxygen: possibly Sulphur, Phosphorus, and others.
POSTERIOR LOBE	$\alpha$ - and $\beta$ -hypoph- amine	
PANCREAS	Insulin	Carbon, Hydrogen, Nitrogen, Oxygen, Sulphur.
GONADS TESTIS	Testosterone Androsterone	Carbon, Hydrogen, Oxygen.
OVARY	The Oestrogens Progesterone	
ADRENAL MEDULLA	Adrenaline	Carbon, Hydrogen, Nitrogen, Oxygen.
CORTEX	Corticosterone	Carbon, Hydrogen, Oxygen.
THYROID	Thyroxine	Carbon, Hydrogen, Oxygen, Nitrogen, Iodine.
PARATHYROID	Not isolated	Probably Carbon, Hydrogen, Nitrogen, Oxygen. Possibly others.
THYMUS, PINEAL BODY, SPLEEN	Endocrine activity uncertain	

## CHAPTER XII

### TRACES

§ 22 THAT many elements, previously thought to be present in food as accidental and inert contaminants, are almost certainly essential to the well-being of those consuming the food has come to be recognized only during this century. Two main causes have contributed to the change of view. First of all, our knowledge of the fundamental effects brought about by minute quantities of certain substances—the hormones, the vitamins, the enzymes, in particular—is of only recent origin. Secondly, the detection of many of the trace elements had to await the necessary refinements of analytical chemistry. Some trace elements, indeed, are not yet to be accurately estimated by chemical means, and the spectroscopist has had to be called in to establish their presence or absence. Spectroscopy, too, has sharpened its weapons, and is to-day capable of a delicacy of detection and an accuracy of measurement not to be foreseen fifty years ago.

§ 23 One word of warning must, in honesty, be given here. Conclusive though the evidence is for the essential part played by several trace elements in the metabolism of animals, including often domesticated farm animals as well as those in the laboratory, direct information as to *man's* requirements is difficult, if not impossible, to obtain. The conclusion that man also needs many, if not all, of these trace elements is almost entirely a matter of inference. The wide distribution of these elements, their detection in the human body when careful chemical analysis has been part of any post-mortem examinations; the general principle that substances essential to the life of one species of mammal are likely to be essential to others; the occasional, though rare, occurrence in human patients of conditions similar to those produced in experimental animals deprived of some trace element, and similarly amenable to treatment

by its addition to the diet—all these facts, and others, make a strong *prima facie* case for holding that man does indeed need these traces.

§ 24 Though it is not possible here to describe, even in outline, the many researches that support these views, it is proposed to give some details of one of them, perhaps the most important, because of the satisfying completeness of the investigations and the general acceptance commanded by the results. It is the story of how copper came to be recognized as indispensable for the formation of hæmoglobin, a story of which the main scene is the laboratory of Professor E. B. Hart and his colleagues at the University of Wisconsin in Madison.

If rats are fed on a diet of milk only, or milk and some highly purified cereal product, like starch, they ultimately die. Supplementing this diet with any or all of the known vitamins in pure or highly concentrated form does not ward off this end. The Wisconsin workers soon found that the immediate cause of the rats' decline was anæmia, a shortage of hæmoglobin and the red-blood cells that carry this pigment. They proposed to call the condition, which is brought about by simple dietary restriction, milk anæmia or, more generally, experimental nutritional anæmia.

The classical, and to us obvious, remedy for anæmia—that is, the administration of iron to the patient—gave very variable results when applied to these anæmic rats. Sometimes they got better, sometimes they did not. The treatment was particularly ineffective with very pure iron, given as one of its salts, the chloride or sulphate. On the other hand, quite small doses of liver were dramatically effective. Liver was known to be a relatively rich source of iron, but this could hardly be the cause of a cure not brought about by iron itself, especially when purified.

§ 25 Hart and his associates therefore next turned their attention to liver. They burnt liver in crucibles and tested the effect of the resulting ash on the anæmic rats. If the ash was fed as such, there was very little result; but if the ash was dissolved in a little acid and

fed in a dilute aqueous solution to the rats, they recovered. This, at any rate, established one conclusion of fundamental importance; whatever it was in liver that cured the anæmia, it was certainly not any of its *organic* constituents—fat or protein or carbohydrate, for example—for all of these are entirely destroyed during the process of incineration. The next question to tackle was, which of the inorganic constituents in liver-ash, soluble in acids, was the curative agent.

§ 26 To settle this, they did something so simple that many other workers probably want to kick themselves for not having thought of it. When the analytical chemist is confronted with a solution of metallic salts—that is, of metals or their oxides dissolved in acid—and wants to find out which metals are present, he follows a very old-established procedure for dividing up the metals present into groups by the use of suitable reagents. He makes his solution very acid with hydrochloric acid, and the relatively insoluble chlorides of silver and lead are thrown down as precipitates, which he can separate from what remains in solution by simple filtration. He then passes hydrogen sulphide gas through this solution, and all the metals present with sulphides insoluble in hydrochloric acid are thrown down as those sulphides. Other reagents are successively employed, and then by similar procedures each group is itself subdivided until every individual metal is recognized by the use of some reaction distinguishing it from those that have accompanied it.

This is exactly what the Madison investigators did with their solution of liver-ash. But instead of wasting time—and animals and material—by separating *all* the metals present, some of them in minute quantities, they carried out preliminary tests on the precipitates in each group. The insoluble chlorides, brought back suitably into solution, were inactive; so were iron, aluminium, zinc, manganese, cobalt and nickel, which are thrown down together in “Group III” as hydroxides. The alkali metal group, in which calcium, strontium and barium occur, also proved useless. But the “Group II”

precipitate, in which are to be found the sulphides of mercury, copper, arsenic and cadmium, was remarkably effective. A solution of this precipitate in acid, diluted with water, could cure, and would prevent completely, the development of milk anæmia in Hart's experimental rats, if given together with a salt of pure iron.

§ 27 From this point on, developments were rapid. None of the metals in this group, whether separated as sulphides from liver-ash, or taken from the laboratory reagent bottle as soluble salts, were of the slightest benefit to the anæmic rats—except copper. That element worked every time. It mattered not whether the chloride or the sulphate or the nitrate was used; extremely small quantities of any of them were completely effective in counteracting the evil effects of the milk diet. With these traces of copper the rats grew nearly normally, and their blood carried its full complement of red cells and of hæmoglobin. These results were obtained with copper *and* iron, though pure iron by itself, as we have already seen, was useless.

§ 28 Milk, especially cow's milk, never allowed to come into contact with iron or steel vessels, is a poor source of iron and is also practically devoid of copper; both these elements are essential for the formation of normal blood. The reason why the young rat could manage fairly well without any iron at all to supplement its milk ration, but was doomed without copper, was subsequently found to be that it is born into the world with sufficient reserves of iron in its liver to carry it over the early stages of its life. But this iron cannot be mobilized in the absence of copper, which is present neither in the infant rat's own body nor in the milk diet. It is, however, not easy to understand how the normal rat gets its copper before it is weaned; possibly it begins eating independently in sufficient time to prevent symptoms of copper deficiency. This phenomenon of an "iron reserve" in the liver of the new-born has been found to be quite general: even the infants of ill-nourished mothers seldom show signs of anæmia before the age of six months. The reserves, however, are not only soon exhausted, but



are built up at the expense of the mother's own supply. That is the reason why modern nutritionists can hardly emphasize sufficiently the need to secure a high iron intake for expectant and nursing mothers.

§ 29 For the reasons already mentioned, we cannot be absolutely certain that man requires copper as the rat does. Fortunately, however, most foods that are sources of iron are also sources of copper, and medicinal iron salts, too, are nearly always accompanied by traces of the other metal. The Wisconsin workers found that the optimum ratio of copper to iron was only about 1 to 50. There is some evidence that certain cases of nutritional anæmia in the human subject respond better to treatment with copper plus iron than with iron alone, but certainty as to the part played by copper in human nutrition has not been reached. It is practically impossible to arrange for human beings, even if they agree to become experimental animals, to receive a copper-free diet, and without establishing the effect of such a diet we cannot get a final answer to the question. That man does need copper remains a conclusion based upon indirect evidence, but the weight of that evidence is impressive to the point of conviction.

§ 30 Our knowledge of man's need for other trace elements is less firmly grounded. But there are several of which it can be asserted with confidence that the experimental rat cannot live normally without them. Manganese, for example, can only be completely withdrawn from their diet at the expense of their reproductive system. There is also evidence that manganese can play a part similar to copper in the formation of red-blood cells, but this has been disputed by some investigators. It has, however, been pointed out that the wide distribution of manganese, and especially its regular occurrence in the reproductive organs of animals and plants, is a further reason for regarding it as an element essential to normal health. Attempts have been made to detect bodily changes due to diets containing quantities of certain other elements well below what is normally found in foods: no clear evidence that laboratory animals

need zinc, nickel, silicon or fluorine has yet been obtained experimentally.

§ 31 One metal, however, is of unusual interest in this connection. Some years ago it was found that adding cobalt to the diet of young rats produces a large increase in the number of blood-cells, a condition known as polycythemia. It has not, however, been possible to show clearly that deprivation of cobalt, normally present in diets only in minute quantities, has any adverse effect, whether on blood composition or otherwise. Nevertheless it has been proved beyond the slightest doubt that one species of animal—the sheep—is incapable of living without it.

In some parts of Australia and New Zealand an apparently incurable disease in sheep was found to be associated with soils that contained only a negligible quantity of cobalt. This correlation was established only after much painstaking research and after many false clues had been arduously followed. The disease, called “pine” or “coastal sickness” and by other names, was attributed to iron shortage, to infestation by parasitic worms and to various causes now known not to be the real source of the trouble. The administration of cobalt to these sick animals has an almost incredibly dramatic result. As little as one milligram a day will restore to health a sheep that is literally a bag of bones, doomed to almost immediate death from this costly disease. In a week or so the sheep is “as good as new”; a similar result can be secured indirectly by dressing the soil with appropriate preparations of cobalt and transferring the sheep to grass growing on the treated soil or even to pasture on which other sheep thrive and where cobalt is presumably present in adequate amount. There is reason to believe that the lack of cobalt in some districts of England—for example, on parts of Dartmoor—is responsible for outbreaks of illness among the sheep there.

The fact that cobalt is indispensable to the sheep but has not been proved essential for the rat is further warning against arguing too readily from one species of animal to another and leaves its necessity to man still an open question.

§ 32 Analytical difficulties have complicated all research in the field of "trace" elements. Even the now-accepted need for manganese took a lot of proving, and was indeed not proved until sufficiently refined chemical and physical technique made it possible to prepare diets almost entirely free of manganese. There is never any possibility of saying that *any* element is absent from a given satisfactory diet: all that the analyst can do is to say that it contains less than a certain quantity—the smallest quantity, namely, that his methods are able to detect. The most we can say of man's requirements for a trace element is either that there is evidence that he needs it or that there is no evidence: the lack of evidence may be qualified by considering the distribution of the element in ordinary foods and in the earth's crust and by other relevant facts.

Progress in this field has been satisfactorily rapid during the last two decades, and no one will be surprised if other elements have to be added to those classified in this chapter as essential to one species or another—namely, copper, manganese and cobalt; sooner or later we may have to add to them zinc, aluminium, fluorine, silicon and even other less abundant elements.

## SECTION IV—VITAMINS

### CHAPTER XIII

#### VITAMINS IN GENERAL

§ 1 To talk, as is often done, of the discovery of the vitamins—whether the credit thereof be given to Hopkins, Funk, McCollum, Babcock, Ragnar Berg, Eijkman, Grijns, or any other of the distinguished scientists who first made laboratory experiments with “accessory food factors”—is to sacrifice historical accuracy to subconscious hero-worship. The pioneers in the field would probably be the first to admit that their early investigations originated in no sudden genial flash of inspiration. An increasing interest in the scientific bases of nutrition accompanied a growing awareness that diet and health were closely connected, and this was itself perhaps in part a reaction against the predominatingly bacteriological view of disease. People were beginning to wonder, moreover, not only at the prevalence of disease despite great advances in social hygiene, and the prevalence of poverty despite the economic triumphs of the Victorian age, but also at the patent correlation between disease and poverty.

§ 2 Those who were well versed in what we have called the “classical” views of nutrition knew that many of its exponents, years before, had hinted at the possible existence of essentials other than fat, carbohydrates, proteins and minerals. There is to-day hardly a book on vitamins—and there are several excellent ones—that does not open with some quotation from an eighteenth-century author, or perhaps even an earlier one, suggesting lines of thought that might well be considered the beginnings of modern vitamin science.

It must, however, always be remembered that speculation may be far removed from knowledge, especially in those sciences that are experimental rather than observa-

tional. For advances in these sciences are at least as much dependent on the state of experimental technique at any time as on the brilliance of their practitioners. And the state of experimental technique in a given science is in turn related to that of the contiguous sciences.

The development of vitamin science in the last three decades has been made possible only by the application of modern methods in chemistry and physics to problems that had themselves been recognized for many generations. Scurvy, beri-beri, rickets and pellagra figure in the chronicles of travellers and explorers since the beginning of world navigation. But micro-analysis, which enables a chemist to assign a formula to a compound of which he has only a few milligrams at his disposal, and spectroscopy, which can detect a fraction of a part of one metal in the presence of a million parts of another, are products mainly of the twentieth century. Why these technical weapons became so sharpened at this particular time is a matter that the student of scientific history will find well worth while pursuing.

§3 The simple question "What are vitamins?" is often asked of those who speak or write on the subject. As will be seen by the readers of the following pages, it is one to which no simple answer can be given. The even simpler question "What is a vitamin?" is almost as difficult to answer with a clear-cut definition. Moreover, to-day a definition in general terms is hardly called for; most readers of the daily and weekly press have an idea, however vague, of the word's broad meaning.

As we shall see, every known vitamin—by which is meant every vitamin that has been isolated in the pure or almost pure state—is a single, clearly-defined chemical compound; this statement is in no way inconsistent with the fact that we have instances of more than one compound having the same vitamin action. The fundamental fact is that all of them are entities in exactly the same way that a fatty acid or glucose or an amino-acid or an iron salt or a hormone is a single chemical entity. They are not "states of matter" or "forms of energy" (save in the ultimate analysis of atomic physics, like

all other substances). They are individual chemical compounds.

If we have stated the fact rather more often than the reader thinks tactful, we would offer him two reasons. First, that there is still so much loose writing in the lay press (and indeed not always only in the *lay* press) about these food constituents as to make it necessary to do everything possible to try to destroy the obscurantism that still largely surrounds them—an obscurantism that breeds charlatanism and dishonest commercialism with terrifying fecundity. Secondly, that we have written of the vitamins as *individual* chemical compounds, and we have done this with intent. By virtue of their individual nature, the vitamins have individual properties. They can in no circumstances whatever replace one another. The man who takes his morning orange-juice because he thereby gets his daily requirements of "vitamin" has failed to grasp this essential point. Apart from the fact that fruit-juices are of nutritional value for reasons other than their vitamin content, orange-juice is a valuable source predominantly of one vitamin. Crates of oranges are useless in the treatment of rickets, for they contain no vitamin D whatever: crates of oranges would be needed to mitigate night-blindness or beriberi, for they are very poor sources of carotene and aneurine. But a few oranges a day will effect the complete cure of all but the most advanced cases of scurvy. And an orange a day will most certainly keep scurvy away from all normal subjects.

§4 There are two loose statements made about the vitamins that call for brief comment here. It is often said that a complete diet will give any individual *all* the vitamins that he needs and further that man is entirely dependent on his food for his supply of vitamins. The first statement is true only if we *define* a good diet for the dweller in a modern industrial city as one containing fish-liver or fish-liver oil: the second statement is equally questionable, for normal man in natural surroundings of fresh air and *sunshine*, where this is not obscured by the urban smoke-pall, is as fully able to

synthesize his own vitamin D as his own thyroxine or insulin.

What constituents of his food are necessary to enable him to effect this synthesis we can hardly even guess, but we know that some of the lowliest vegetable organisms can synthesize sterols, with which vitamin D is closely related, from simple food constituents like glucose. There is also experimental evidence that other animals, such as rats, can synthesize sterols: a laying hen puts out more cholesterol in her eggs and excreta than she eats in her food. True, as we shall see later, man can only "synthesize" his vitamin D from its precursor, pro-vitamin D, which circulates in his blood stream, when this undergoes ultra-violet irradiation in his skin. This is, however, only to put the problem back one stage. The pro-vitamin is itself a sterol and must be synthesized by the animal organism or come from its food. As purely vegetarian animals can never consume food sources of the typical animal pro-vitamin D, which does not occur in plants, such animals must make their own. There is no reason to believe that man is devoid of this ability. In this sense, then, rural man makes his vitamin D for himself and is therefore not solely dependent on his food for his supply of vitamins as such; urban man, and especially the urban child, is likely to suffer from vitamin-D deficiency, and to be prone to rickets even on the best "all-round" diet, unless he receives some medicinal source thereof.

§ 5 This damnable iteration of the facts of vitamin specificity seems to be sadly needed. The quotation below appeared in a daily paper less than twenty years ago. If the doctor has been misrepresented, the sub-editor must be charged with ignorance otherwise to be laid at the medical officer's door. Anyhow, this is the report:—

"Dr. —, County Medical Officer, said he was afraid that the explanation for the large number of school pupils who were receiving after care massage treatment for rickets was due to the diet they were given. They were not receiving sufficient protein or

meat diet. The parents could only buy meat once a week, and at school the pupils got soup, bread, and pudding, but no meat in it."

If that is what he really did say, it shows that his heart may have been in the right place, but that his information was a little lacking. No amount of protein in the diet can have the slightest effect on rickets, and the undoubted ill effects of an inadequate protein supply are entirely distinct from rickets, nor will vitamin D or calcium or phosphorus additions make good the deficiency.

§ 6 The number of vitamins—or groups of vitamins—to-day recognized as occurring in foods cannot be stated exactly. Several have been isolated, analysed and synthesized in the laboratory—and even on a large scale in the chemical factory. Then there are a few vitamins for the existence of which, and their necessity for mankind, there is strong, sometimes incontrovertible evidence, although they have not been obtained in a state of purity—or even approaching purity. For this reason it cannot be known whether any particular one of them is indeed only one; it has happened several times in the history of vitamin research that a condition first thought to be due to a single deficiency has later been found to be of multiple origin—and it is only by the effects of their absence that the vitamins have come to be characterized.

§ 7 There are two further complications that make it impossible to answer in one word the question "How many, vitamins are there?" We know of at least two substances that are not strictly speaking vitamins, but are turned by the body into vitamins. They are frequently spoken of as pro-vitamins. Carotene is turned into vitamin A somewhere in the body; it is certainly not the same as vitamin A, for the chemical constitutions of both are known, and they are different. Again, the skin contains pro-vitamin D, a substance without any known direct physiological activity: under the action of certain ultra-violet radiations, natural or artificial, it is converted by the body into vitamin D.

Further, we know of several instances where two



or more substances, different though closely related in chemical constitution, occur in plant or animal products and have the same vitamin action. Also, a number of artificial substances have been made in the laboratory having the same action as naturally-occurring vitamins, if to a less extent, but differing slightly in constitution from them.

These facts will be discussed in a little more detail in the following chapters, but they afford another warning against the glib generalizations in which the subject is all too rich. In no circumstances is it justifiable to make artificially simple a position that is naturally complex, if this can be done only at the expense of accuracy. A refusal on scientific grounds to answer a particular question is not necessarily an admission of weakness; as often as not it is unavoidable because the wrong question has been asked!

§ 8 There are, however, a number of questions that may legitimately be asked, because they can be scientifically answered—how the existence of a particular vitamin has been recognized, how its quantities in different sources have been estimated, how it has been purified, what constitution has been assigned to it, whether this constitution has been confirmed by synthesis, what are known to be the consequences of its absence from, or deficiency in, a human dietary and whether it has any uses besides making good those deficiencies when they are liable to occur or have occurred.

It is impossible in this book to answer each of these questions for all of those vitamins about which the relevant information is available. We must therefore have recourse to a compromise. We shall try to give the answer in some detail to each one of these questions for at least one particular vitamin and content ourselves, if not the reader, by summarizing the answers for the others—where the answers are known or suspected. In the next chapter, therefore, we shall discuss the question of recognizing a vitamin's existence, with special reference to vitamin A. In the following chapter we shall consider the comparative and absolute measurement of vitamin C

(ascorbic acid). Then we shall describe some of the chemical work on vitamin D (calciferol and certain allied substances). After that we shall consider the various deficiency diseases that are now held to be solely or primarily due to shortage of one or other vitamin. In the last section of this book certain aspects of human nutrition will then be briefly discussed.

## CHAPTER XIV

### DETECTING VITAMINS

§ 9 To treat this subject historically would involve considering many irrelevancies and following side-tracks that have led away from the main road of advance. They are inevitable in an experimental science, especially one that is concerned with the complex processes of living organisms. There are so many unknown factors that cannot be controlled by the investigator just because they *are* unknown. We shall, therefore, occasionally find it necessary to illustrate the arguments in this and subsequent chapters by describing experiments that may not have been carried out in the order or manner set forth. Nevertheless, they will all be, as far as it is found possible to make them so, accounts of what would have happened if the experiments had indeed been made and of what would happen if they were made to-day.

§ 10 Let us suppose we have available some young rats of the kind preferred by workers in nutritional laboratories. We want to see if it is possible to bring them up on a simplified diet of purified substances—satisfying the requirements of “classical” nutrition and as complete as those requirements involve. It would be composed roughly as follows: first there would be some “good” protein—say the casein that is precipitated by acid from skim-milk. The casein would have been submitted to special treatment for removal of any butter-fat that may have escaped separation from the skim-milk, for we want to control each major dietary constituent separately, and we intend to look after the fat-content next. This we shall do by including 10 per cent. or so of a good vegetable oil—say arachis (pea-nut) oil. We shall add a carbohydrate, cane-sugar or starch, and a mixture of mineral salts, not forgetting the trace elements, iron, copper and the rest, which we discussed in the last section of this book.

§ 11 This diet can conveniently be made into a paste with water and may be offered to the young animals *ad libitum*, to make sure that partial starvation does not complicate our experiment. On such a diet it would be found that the animals, though taking enough food for calorific requirements and perhaps for some growth, would entirely cease growing in a week or so, or even in a few days, and would all be dead in three or four weeks. "Classical" nutrition is not enough, evidently; the rats require something besides protein, fat, carbohydrates, minerals and water. If we add to the diet 10, or even 5, per cent. of dried yeast, the difference will be phenomenal. The rats will grow rapidly, remaining lively and healthy-looking for several weeks. The yeast has clearly supplied something besides its content of fat and protein (both of which occur in the yeast plant), for these were already present abundantly in our "basal" diet. But even the yeast will not suffice for continued health and life. After a few weeks—eight or ten or twelve, depending partly on circumstances connected with the nutrition of the young rats' mothers—growth will again cease; the animals begin to look sick, and a suspicious redness appears round their eyes. This is followed by a discharge of pus and blood from the eyelids, and bacteriological examination of this discharge will reveal that the outer surface of the eye is heavily infected with micro-organisms. Some of the animals will develop pneumonia, to which the tame albino rat is rather prone; these will undergo a catastrophic fall in weight, ending fatally in a day or so. Others may merely lose weight slowly, but all will decline, and not a rat will be left at the end of three or four months.

§ 12 If, however, we take some of these animals before they are too far gone in their decline, and give them a single drop of medicinal cod-liver oil each day, a change will occur very nearly as dramatic as that brought about by yeast. The eye trouble will clear rapidly, and the eyes will become quite normal, unless the infection (known as xerophthalmia) has proceeded too far, in which event the animal may be left blind in one or both eyes, though

the infecting organisms will have disappeared. For the most part the animals will begin growing again and may live to a good old age on the original "basal" diet with its additions of yeast and cod-liver oil. In two respects, however, they will differ from normal rats and resemble their less fortunate brethren whom we may not have tried, or may have failed, to cure with cod-liver oil. They will be more or less permanently under-weight for their age, and post-mortem examination of their various organs will reveal the presence of many abscesses, now perhaps healed, in mouth and alimentary canal, as well as other lesions in kidneys, liver and so on. These abscesses and lesions would be found much more prominently, however, in the animals that died before any cod-liver oil was added to their diet.

This effect of cod-liver oil could also have been demonstrated prophylactically. That is to say, had we added the cod-liver oil to the basal diet at the beginning of the experiment (along with the yeast, of course), then there would have been no xerophthalmia, no slackening or cessation of growth, no deaths from pneumonia, no internal abscesses or lesions—nothing, in short, to distinguish the rats from those brought up on a complete diet of natural foods. (This statement needs slightly qualifying; it is extremely difficult, even to-day, to devise a semi-synthetic diet that gives just as good growth as, say, one made up of kitchen scrap or one containing milk and meat and green leaves. Investigations in my own laboratory, however, suggest that the difference may be almost entirely one of palatability and that the experimental diet and the diet of natural foods would be equally effective if the rats would eat as much of the former as of the latter. Broadly speaking, however, the supplemented basal diet can be considered as complete for the rat as present-day knowledge allows us to make it.)

§13 It is thus clear that the cod-liver oil contains something essential to the rats' health and something that it needs in relatively small quantities, for we know that the one drop of cod-liver oil, about 20 milli-

grams or  $\frac{1}{2}$  grain, consists very largely of fat, in nutritive properties not unlike the arachis oil abundantly present in the basal diet.

Now the use of cod-liver oil to cure rickets in the human being has been recognized in Western Europe and the United States for several decades, and on the coast of Norway, where it might almost be counted as a common food, for centuries. This led many investigators to the idea that the same constituent in cod-liver oil might be responsible for curing (and preventing) rickets in the human infant as for the dramatic effect on the deficient rat. Two sets of experimental facts, however, demonstrated that this could not be true.

§ 14 By choice of a suitable basal diet, rather different from the one described in § 10, it is possible to give to newly-weaned rats very severe rickets in the course of three or four weeks. This is cured or prevented by quite small quantities of cod-liver oil: in this and many other respects the condition is remarkably similar to the clinical rickets of the human young. It is called "experimental rickets"; the ability to produce experimental rickets in rats was discovered independently by two different schools of American scientists some twenty years ago and was one of the supreme technical advances in vitamin research.

Cod-liver oil, then, besides curing human rickets, will also cure experimental rickets in rats as well as the condition of deficiency associated with xerophthalmia. It looks more than ever as if the same factor were at work in all three cases. This appearance was soon dispelled. It was found that other substances, notably green vegetables and butter-fat, entirely or almost entirely ineffective in the treatment of human *or* experimental rickets, were as well able as cod-liver oil to cope with the deficiency cured by cod-liver oil. It was also found that cod-liver oil, after it had been heated to a moderate temperature and had air blown through it the while, though it remained a highly effective anti-rachitic agent, was quite incapable of adequately supplementing our original basal diet plus yeast.

There was only one conclusion to be drawn from these observations. There must be in cod-liver oil *two* dietary substances, one of which (or something very like it) is also present in butter-fat and green leaves and deals specifically with the deficiency condition that involves xerophthalmia; the anti-rachitic substance is clearly something different, is generally absent from butter-fat and leaves and, incidentally, is much more resistant to the action of heat and aeration. This second substance is now called vitamin D—the anti-rachitic vitamin. The other is known as vitamin A—the growth-promoting, anti-xerophthalmic vitamin. At one time there was a tendency to adopt the suggestion to call this the “anti-infective vitamin,” a tendency fortunately now abandoned. This is a good thing if only for the reason that increased liability to infection is also associated with shortage of other nutrients, including several of the vitamins. Moreover, there seemed to be implied in the term a suggestion that piling up the intake of this particular vitamin above the level usually necessary for maximum health would confer some extra special resistance to disease. Such a view is not merely controversial, but one to be greeted with legitimate scepticism. There is little evidence that increasing the intake of any nutrient above the *smallest* amount necessary to give the *best* effect can produce any additional benefits. This is not, however, to deny the possibility that the therapeutic administration, under proper medical direction, of very large doses of certain vitamins may produce effects different from those produced by ordinary doses in ordinary diets and perhaps not at all clearly related to those normal physiological effects.

§ 15 In similar ways, using basal diets so constituted as to bring about specific deficiencies in suitable animals, the existence of other distinct vitamins can be demonstrated. Cod-liver oil, as we have already mentioned, can be shown to contain a second vitamin different from vitamin A and quite unable to depute for it. Vitamin D, as it was called when it had been clearly differentiated from vitamin A, is the substance—or group

of substances—capable of preventing or curing “experimental rickets” in rats, as well as an almost equally clear-cut abnormality in young growing chicks; this condition is generally referred to, rather colloquially, simply as “leg-weakness.”

§ 16 Diets can be devised that, though ample in their contents of vitamins A and D, are still incapable of supporting growth in young rats. Yeast contains a number of other vitamins. These together were called vitamin B, before it was established that more than one vitamin must be present to account for its effect on rats receiving all the major constituents of diet, the essential minerals and vitamins A and D. One of the yeast vitamins, vitamin B<sub>1</sub>, now called aneurine, is also necessary for pigeons; these birds—and other species on which similar experiments have been conducted—develop a characteristic set of nervous and muscular symptoms if fed on a diet consisting entirely of grain deprived of its “bran” or germ. The B<sub>1</sub>-deficient pigeon goes into a curious condition of spasm, with its neck bent back, known as opisthotonos, and is in such a state of unco-ordination that it will turn involuntary “cartwheels” in its cage. A few grains of yeast, or a few drops of a yeast extract suitably prepared, will effect an astounding change, if forced into the crop of such a polyneuritic pigeon. In less than an hour the bird looks and behaves like a normal animal.

§ 17 Besides aneurine (vitamin B<sub>1</sub>), which is called thiamin in the U.S.A. and also by many British and foreign workers, there is present in yeast (and liver and cereals) a group of water-soluble vitamins, comprising with aneurine the original “vitamin B” of the vitamin pioneers and called vitamin B<sub>2</sub> when its difference from aneurine was clearly established. Later it was apparent that this in turn was not a single substance, and it became known as the “vitamin B<sub>2</sub> complex.” Its outstanding property—anyhow as observed in animal experiments—was its ability to restore normal growth rates to animals (*e.g.* rats and chickens) that had received for some weeks diets substantially free of it. Then it



was discovered that this "growth-factor" was a single chemical substance, first called lactoflavin, because it had been found that milk was an especially rich source of it. Indeed, the first pure samples of this vitamin were made from whey. Subsequently it was discovered that it contained in its molecule the same structure as the comparatively rare sugar *ribose*, and that it was chemically identical with physiologically similar substances found in liver, malt extract and urine. The more general name riboflavine was proposed for it and has now received general acceptance. Because of its major contribution to the growth-promoting properties of the "vitamin B<sub>2</sub> complex," or because it was the first definite compound to be identified in the complex, or for both reasons, some workers still refer to riboflavine as "vitamin B<sub>2</sub>." This is historically incorrect and leads to quite unnecessary confusion: moreover, it transgresses the rule, accepted by most responsible workers in the field, that the alphabetical designation of a vitamin should be abandoned as soon as it has been chemically identified and given a name.

For this reason also, the habit of a few—fortunately only a very few—workers, who identify nicotinic acid with vitamin B<sub>2</sub> is equally, or more, to be deprecated. We noted in Chapter II that this compound, although an essential nutrient for man, dog and pig, can be synthesized by the rat (as also apparently by the mouse and possibly many other species). Moreover, as vitamin B<sub>2</sub> was historically the non-aneurine portion of the old yeast "vitamin B," and as the existence of "vitamin B" was established by its effect on rats, how can we include in it a substance that rats don't need? Furthermore, since nicotinic acid was perfectly well known to chemists for years before its nutritional properties were discovered, there is not the slightest justification for calling it by anything but its "familiar" chemical name or by its more strictly scientific and descriptive name, pyridine- $\beta$ -carboxylic acid.

Both riboflavine and nicotinic acid play fundamental and essential parts in the living processes of individual cells,

possibly of every cell in every living plant and animal—almost certainly of every cell in which carbohydrate is “burnt,” that is to say, oxidized with release of energy, ultimately to give carbon dioxide and water. It is therefore of particular interest to note that, as already mentioned, nicotinic acid shortage leads, if complete, to a clean-cut well-recognized deficiency disease—pellagra—with its well-known “three D” symptoms (diarrhœa, dermatitis and dementia) and its fatal end, if curative steps are not taken in time. Riboflavine deficiency, on the other hand, leads only to certain symptoms rather difficult to characterize (inflammation of tongue and cornea, fissures in the corners of the mouth and at other epithelial junctions), and by no means all of them entirely specific to this one cause. Nevertheless, there is little doubt that riboflavine deficiency, at least of a mild kind, can be found by expert diagnosticians, especially if they look for it in the worst-paid sections of industrial or colonial workers. Moreover, there seems to be evidence that pellagra—in the sense of nicotinic acid deficiency—is frequently complicated by symptoms of riboflavine deficiency, so that both vitamins are needed to restore normal health, when it is not too late to achieve this.

My friend Dr. Isaac Frost pointed out to me once that an interesting change had come over the relationship we recognize between vitamins and deficiency diseases. Formerly we invariably identified a vitamin by the pathological conditions that it would cure or prevent: now we tend to identify deficiency diseases—or even the specific symptoms—by the vitamins that will prevent or cure them! It is much easier to define pellagra as “nicotinic acid deficiency” than in any other way and to confirm its diagnosis by the effects of administering nicotinic acid. Absence of riboflavine from the diet (aribo flavinosis) or its presence in inadequate amounts (hyporiboflavinoses), indeed, are almost impossible at present to diagnose with certainty save by the results that follow from increasing the patient’s intake of riboflavine.

It is not possible here to mention all the constituents of the vitamin B<sub>2</sub> complex that have been isolated and

chemically characterized, or to refer individually to those of them believed or suspected to have vitamin-like functions. Two of them, however, are of sufficient importance, being essential to many forms of plant and animal life, to be noted here: they are known respectively as pantothenic acid and biotin (at one time called vitamin H). Chemically they are very different from any of the compounds so far mentioned in this chapter, and serve once more to remind us how heterogeneous is the "class" of substances called vitamins, both in molecular make-up and in biological properties.

Yet another substance present in the "vitamin B<sub>2</sub> complex" has been variously known as vitamin B<sub>6</sub>, adermin and pyridoxin—the last being the U.S. name and the one now in most general use. This has certain chemical affinities with nicotinic acid, but, unlike that compound, it is needed by rats which, if it is absent from their diet, develop a characteristic and very severe dermatitis. Two closely related compounds, with similar vitamin properties, are pyridoxal and pyridoxamine. Finally, a compound known for many years to the organic chemist as *para*-aminobenzoic acid has now been clearly established as an essential growth-factor for many different kinds of plants and animals, ranging from bacteria to mammals.

Nothing is yet known about man's requirements for *para*-aminobenzoic and pantothenic acid or pyridoxin, but most workers in the field would include these vitamins among the nutrients essential to man, not only by analogy with laboratory animals, but also because of their widespread occurrence in plant and animal (including human) tissues. Biotin deficiency, on the other hand, has been experimentally induced in human volunteers, in whom it was rapidly relieved by administering very small amounts of the pure vitamin. One case report in the medical literature, moreover, gives an account of a condition that seemed to be, at any rate in part, due to shortage of biotin in the diet and that was, at any rate in part, much improved by giving the patient biotin.

The five members of the "vitamin B<sub>2</sub> complex"

described above—riboflavine, nicotinic acid, pantothenic acid, biotin, and pyridoxin—are all substances of well-established chemical structure. All have been synthesized in the laboratory—four of them on a manufacturing scale, as of course has also aneurine (vitamin B<sub>1</sub>). The large-scale preparation of biotin is likely to be accomplished as soon as there is evidence of any considerable need for it in medicine.

§18 Green vegetables and fruit juices—particularly those of the lemon and orange and also black currants—contain a vitamin that is not needed in the rat's food, for this animal seems to be able to make its own. But the guinea-pig dies without an adequate supply of vitamin C, or ascorbic acid, as it is now called. This is the anti-scorbutic vitamin, for the condition developed in its absence by the guinea-pig is "experimental scurvy."

Another substance in citrus fruits and many food sources of ascorbic acid, though the distribution is by no means always parallel, has been called vitamin P: without it the capillaries in the guinea-pig's skin develop a marked weakness, so that cutaneous hæmorrhages and bruising are very easily produced. A similar condition has been described in man: like the capillary fragility of the guinea-pig, it can be prevented or cured by foods containing vitamin P, or by concentrates prepared chemically from these foods, but is unaffected by pure ascorbic acid.

§19 Among the established vitamins must also be mentioned vitamin E, the anti-sterility factor for rats. Without this the male rat soon loses permanently all power of producing living spermatozoa and therefore of effective mating; the female rat receiving a vitamin E-deficient diet, on the other hand, is capable of mating, but not of giving birth to a live litter. Instead, the embryonic animals undergo disintegration and are reabsorbed into the maternal tissues—a process called gestation-resorption. Restoration of vitamin E to the diet of the female rat enables her once more to produce full-term living young; vitamin-E deficiency in the female rat has been held to differ fundamentally from the male in its reversibility. There are, however, some

reasons for thinking that the effects on the female organism may be more lasting than has hitherto been believed; it will be recalled that the lesions of a rat that has been deprived of vitamin A are still present in animals apparently cured by restoration of the vitamin to their diet.

§ 20 Vitamin K—the antihæmorrhagic vitamin of Dam and Almquist, working independently in Denmark and California—is quite different in chemical structure and in physiological properties from the substance, or group of substances, provisionally called vitamin P. In its absence from the diet the blood of the deficient animal loses its ability to clot, and this may easily lead to death following an external or an internal injury. Even if vitamin K is present in the diet, serious disturbances of fat absorption, due to anatomical or other causes, may prevent absorption of the vitamin and thus lead to the same condition. Two forms of vitamin K occur in nature, one predominantly in plants ( $K_1$ ), and the other in animal sources ( $K_2$ ), according to some authors, but I do not consider that this distinction is very surely grounded. It is, however, now of little more than academic interest, for the use of vitamin K in treatment has been completely replaced by that of a simpler, though closely related, compound of synthetic origin. This substance is chemically described as 2-methyl-1 : 4-naphthoquinone, and is made by oxidizing a well-known coal-tar constituent (2-methylnaphthalene). It has all the biological properties of vitamin K with certain advantages for therapeutic purposes: it has been given the names of menaphthone and menadione in the British and U.S. Pharmacopœias respectively.

§ 21 For the moment, this list of the vitamins must suffice; we shall later have to consider their relationship with human health and particularly with certain clearly defined “deficiency diseases.” It may, in passing, be mentioned that it is often the practice to divide the vitamins into two main groups—the fat-soluble (vitamins A, D, E and K) and the water-soluble (the members of the “vitamin B complex” and vitamins C and P). This division is, however, of little interest except

to the chemist, who, in attempting to isolate or manufacture vitamins, will want to know as much as possible of their properties, and to the pharmacist, who, in dispensing medicinal preparations of them, will have to consider suitable media for their presentation or "exhibition," as he sometimes calls it, preserving a rather quaint term from medical phraseology of a bygone age.

In addition to the recognized vitamins already mentioned there are several other claimants to inclusion in the class—or collection, as it might be less question-begging to call it! Some of these are compounds of known structure but disputed physiological action: others have been hypothesized to explain results obtained in laboratory experiments, but have not been isolated or purified. Still others have been separated as substances that are pure or approaching purity, but in quantities insufficient to allow of their exact chemical or biological characterization. Among those in the first group may be mentioned inositol, a sugar-like alcohol, and linoleic acid, an unsaturated fatty-acid, abundantly present (as glyceride) in linseed oil, and choline, an organic base. The nature of the second and third groups is such as to make it impossible to specify their members in chemical terms, but they run into at least a dozen, if not twice that number. There is plenty more work to be done in this field, despite the dramatic advances made in it during the twentieth century. Until this work is further advanced we cannot legitimately even speculate about the part these substances may play in human health.

§ 22 The recognition in cod-liver oil of some specific dietary constituent, present in quantities that had eluded ordinary chemical analysis, raised immediately a number of questions. What kind of a substance was it? Could it be separated from the known constituents of cod-liver oil—the fatty acids and glycerol, for example—which were certainly incapable of curing or preventing xerophthalmia? Could tests for the vitamin be found that would make investigators independent of laborious and costly animal experiments? And what other animal or vegetable sources contained the vitamin?

It was very soon found that the vitamin was associated with the unsaponifiable portion of the oil, to which we have already made reference earlier in this book. Since this portion of a normal cod-liver oil is only about 1 per cent. of the weight of an oil, it follows that it must be some hundred times as active, weight for weight, as the oil itself. In other words, an effect produced by way of prevention or cure with, say, 20 milligrams of oil could be produced with only  $\frac{1}{100}$  milligram of unsaponifiable matter, assuming that this can be separated from the rest of the oil without any destruction of the vitamin.

By removing from the unsaponifiable matter the whole of the sterols, which show no vitamin-A activity whatever, the activity of the concentrate could be once more increased; sterols make up approximately half the unsaponifiable matter, so that their removal about doubles the activity.

§ 23 Research along these lines was facilitated by two discoveries. First of all, cod-liver oil was found to give, under appropriate conditions, an intense, though rather evanescent, blue colour with antimony trichloride, and the depth of the colour went roughly parallel with the vitamin-A activity. This reaction enabled the chemist to follow up the fractionation of the vitamin and to avoid wasting time by biological tests on any portions that do not give a positive antimony trichloride reaction.

Next it was discovered that the vitamin, or something very closely associated with it, absorbs a certain wavelength in the ultra-violet part of the spectrum, making possible its detection by spectroscopic analysis. This was the first introduction of spectroscopic methods into vitamin research; these have played a part also in elucidating the distribution of properties and structure of almost all those other vitamins about which we possess detailed chemical knowledge.

§ 24 With the "blue test" and the spectroscope at their disposal, biochemists soon discovered that the livers of other fish than the cod contained oils much more suitable for investigation, as they were far richer sources of the vitamin than cod-liver oil. Whereas a relatively

rich cod-liver oil seldom contains, as we now know, more than one part of the vitamin in two thousand of oil (one part in ten or twenty of the unsaponifiable matter), some halibut-liver-oils, though by no means all, were found to contain as much as 2 per cent. ; the unsaponifiable portions of these oils, however, are not proportionately richer, as they constitute nearer 5 than 1 per cent. of halibut-liver oil. Even so, chemists were able to prepare sterol-free concentrates of halibut-liver oil containing as much as 60 or 70 per cent. of the vitamin. These concentrates, submitted to further careful fractionation, finally resulted in the isolation of a pale yellow, viscous oil that defied further subdivision and was some three or four thousand times as active as ordinary cod-liver oil. This product was for several years accepted as the "pure" vitamin, but further investigations showed that an even more active fraction could be separated. The technical difficulties for those working in this field mostly derive from the facts, first, that the highly purified vitamin is a rather unstable substance, and secondly, that to establish the purity of a non-crystalline compound is one of the hardest of tasks for the chemist. Recent work in the United States has led to the production of pure and crystalline vitamin A; the crystals melt to a viscous oil at well above ordinary atmospheric temperatures.

§25 Before we leave the subject of vitamin A we must record one other fact of very great importance. This vitamin is an exclusively animal product, occurring most abundantly in liver oil or fat, whether of fish, bird or mammal, but also present in small quantities in other parts of the body. In particular it is secreted in the fatty portion of milk; summer butter is an important dietary source. Plants, on the other hand, contain, in relatively small quantities by weight, a bright red pigment—carotene—often masked by the simultaneous presence of green and yellow pigments. Carotene is chemically distinct from vitamin A, but closely related to it. The animal body can convert carotene to vitamin A, probably in the intestine, and store the resultant vitamin A in the liver. Carotene can completely replace vitamin



A, not only for the experimental rat, but also for the chicken, the pig, the cow and almost certainly for other vertebrates, including man. That is why fruitarians do not die of vitamin-A deficiency. It is, however, stated that carnivores have no power to convert carotene into vitamin A—quite a subtle economy of metabolic effort! Milk-fat (butter) contains both carotene *and* vitamin A, in proportions that vary with the breed of cattle and with the cows' diet. The carotene is all derived from the cow's food, chiefly from pasture; that is why summer milk contains more vitamin-A activity (that is, the summed activity of carotene and vitamin A proper) than winter milk. A yellow butter—to which no artificial colour has been added—is certain to contain carotene in useful amounts, but a pale butter from a different breed of cow may be an equally good source, or even a better one, if its deficiency in carotene is counterbalanced by a larger amount of vitamin A, which does not affect the appearance of the butter at all. At best, however, summer butter seldom has more than one-twentieth of the total vitamin-A activity of an ordinary cod-liver oil. The relative efficiencies of vitamin A itself and of the pro-vitamin carotene as a source of vitamin A have turned out to be unexpectedly difficult to assess, and nutritionists are still not prepared to dogmatize on the matter. As a very rough approximation, however, it can be taken that, weight for weight, vitamin A in foods (and medicines) is some four to six times as effective as the most active form of carotene.

## MEASURING VITAMINS

§26 It is not possible to draw anything but a quite arbitrary line between detecting and measuring vitamins. When we detect a substance by its action on a living organism, we are in fact making a measurement. We may give the organism more than it needs for the production of a given effect, and in that sense our measurement is indeterminate in one direction. But we have certainly established the fact that this particular effect does not need for its evocation *more* than the particular amount of the substance used. And that is a measurement.

In a series of experiments, however, we could gradually cut down the amount of the substance until we had also found out what is the smallest amount that can produce the given effect. We should thereby have conferred a further degree of exactitude on our measurement. As soon, however, as we begin to make this kind of serial experiment we are met with a real difficulty. Suppose we have thereby established that, say, 1 millilitre (about  $\frac{1}{8}$  oz. or a third of a teaspoonful) of lemon-juice is insufficient to prevent one particular guinea-pig from getting "experimental scurvy" on a diet devoid of green-stuff, while 2 millilitres is sufficient to protect another guinea-pig, are we justified in concluding that, say, about  $1\frac{1}{2}$  millilitres will be just enough to protect all guinea-pigs whatsoever?

§27 The answer to this question, as to so many others, is to be found only by further experiments. In this instance we must follow the same fundamental principles that we have already discussed in describing the methods for detecting vitamins. First, we must get our basal diet right. We will add to the oats and bran, on which we know *all* guinea-pigs develop scurvy, some baked milk-powder; the vitamin A present is necessary to replace the carotene in the green leaves, on which a

guinea-pig depends for its vitamin C as well. The baking is to destroy the small amount of vitamin C that is now known to be present in raw milk and in certain kinds of dried milk. Thus our experiment will not be complicated by the superposition of a vitamin-A deficiency on the experimental scurvy. On this diet, then, of oats, bran and baked milk-powder the guinea-pig stops growing in three or four weeks and soon develops the weak hind-legs, the sore gums, the hæmorrhages under the skin and the softened bone-junctions that characterize experimental scurvy and resemble very closely the symptoms of human scurvy.

Moreover, if we give to other guinea-pigs the same basal diet with supplements of cabbage or lettuce leaves or of orange or lemon juice, they will remain normal and healthy. We have, in fact, shown the diet to be satisfactory for our purposes by carrying out "negative control" and "positive control" experiments with it. We can now proceed to investigate the problem of the *minimum* curative dose.

§ 28 A dozen or so of young growing guinea-pigs are allowed to eat freely of the basal diet (and water, normally derived from the now missing green leaves). When they have all ceased to grow and begin to show clear signs of scurvy, we will give every one of them  $1\frac{1}{2}$  millilitres of the lemon-juice that we are trying to test for its *anti-scorbutic activity*—that is, for its content of ascorbic acid (vitamin C). We shall then find that some of the animals—two or three, perhaps—recover completely, another half-dozen or so cease to lose weight, but do not grow normally, and are still far from well, while the rest die, though certainly not as soon as if they had received no lemon-juice. What, then, is the answer to the question, "Is  $1\frac{1}{2}$  millilitres of the lemon juice a minimal curative dose for experimental scurvy in guinea-pigs?"

The answer is that there is no answer to the question; this is just one of those instances when the wrong question has been asked! The dose of lemon-juice used is sufficient, or more than sufficient, to cure some guinea-pigs and quite inadequate to cure others. Clearly, to

express the vitamin-C content of the lemon-juice in quantitative terms, we must find some way of getting over the ever-present difficulty of individual variations in the test animals.

§29 Indeed, there are two problems to solve here, rather than one. First we have to find a method of measuring the response of each individual animal to a dose, and then we have to consider how best to express the response of a group of animals, taken as a whole, to any one dose. In the kind of experiment described above we shall find that a suitable dose of lemon-juice makes some animals increase in weight more than others. If the dose is too low, no animals will gain at all, and the effect of the dose on the group will be nil; if too large a dose is chosen, all the animals will show the maximum growth possible. Such a dose may clearly have *any* value above a certain amount and is too large for any kind of comparative measurement. But some intermediate dose will permit of sub-maximal growth; doses of that kind must always be used for measuring physiological activity by tests on animals.

§30 Roughly speaking, the most we can make these guinea-pigs gain in weight is about 20 grams a week. The *smallest* dose of lemon-juice able to bring about this result will vary from animal to animal, but there will be a certain dose that produces this effect on all guinea-pigs, as obviously will all larger doses. If we give to a group of guinea-pigs, all receiving the scurvy-producing diet, a dose of lemon-juice about half that able just to produce maximum growth in all guinea-pigs, we shall get the kind of results described in § 26 above. Suppose there are twelve animals in the group. We shall assume that the limiting optimal dose (as discussed above) is 1 drachm (3·5 millilitres) of fresh lemon-juice, so that every animal in the group will be given half a drachm of juice. Then the weekly weight increases of the animals will range themselves something like this:—

1 animal gains 20 grams  
3 animals gain 15 grams

4 animals gain 10 grams  
 2 animals gain 5 grams  
 2 animals, weights stationary.

The average increase in weight for the whole group of twelve animals is  $\frac{20 + 45 + 40 + 10}{12} = 9.6$  grams. We

can now say that for a particular group of guinea-pigs, twelve in number, half a drachm of the lemon-juice produces an average weekly increase in weight amounting to just under 10 grams. There is one way, and only one way, of judging how accurately this weight increase measures the amount of vitamin C in the lemon-juice. We must repeat the experiment with yet more groups of guinea-pigs and again determine the average weekly gain in weight of each group. We shall, in fact, find that this is fairly constant; for some groups it may be as low as 8 grams, for others as high as 12, but most groups will give values round about 9, 10 or 11 grams. In fact, the average weight increases of groups will show fluctuations similar to those shown by individual animals, but much less marked, and the *larger* the groups the *smaller* the fluctuations.

§ 31 For most practical purposes the kind of fluctuations we have attributed to groups of twelve animals will not matter seriously: with groups of, say, twenty animals we should get reasonable results, if we used the same sample of lemon-juice. For this technique we can, therefore, say that the *same* amounts of vitamin C will produce in groups of twenty guinea-pigs approximately the *same* average weekly increases in growth, provided the amount of vitamin C is about half what is just necessary to produce the maximum possible weekly growth increase.

From this it is fairly obvious how to compare the amount of vitamin C in the given sample of lemon-juice with, for instance, the amount in a sample of potatoes or cabbage-leaves or black-currants. We must find the amount of this other source that will also give an average weekly weight increase of about 10 grams, when it is fed

to a group of guinea-pigs receiving a scurvy-producing diet. The larger the group receiving the potatoes or cabbage or currants—that is, the *test* group—and the larger the group receiving the lemon-juice—that is, the *control* group—the more accurate will be our comparison. By statistical methods, moreover, it is possible to analyse our results and accurately to estimate their accuracy.

§32 Such analyses have led to important conclusions as to the best conditions under which to carry out these biological comparisons in order to obtain the maximum amount of precision for a given number of animals. The experiments in both groups should be carried out contemporaneously and the animals in the two groups should be closely “matched”; every animal in the test group should be controlled by an animal as nearly like it as possible. Such pairs of animals should be of the same age, weight and sex; they should come from a uniform (that is, a highly inbred) stock whose diet before the experimental period, including the diet of their mothers during pregnancy and lactation, should be as constant as it can be made. Wherever possible, each pair of animals should come from the same litter.

If these conditions are observed, then the number of animals required to give a certain accuracy of comparison may be very considerably less than, perhaps as little as half, the number required if no attention is paid to the “matching” of pairs in the two groups.

§33 Now, it is clear that such conditions can be fully realized only inside one particular laboratory. Dr. A may find that the growth given by 1 drachm of lemon-juice is identical with that given by 10 grams of potatoes—that is, these potatoes contained about one-third of the vitamin C present in the lemon-juice. But how do these compare with a sample of cabbage-leaves being tested for vitamin-C content in the laboratory of Professor Z? It would certainly be quite wrong to assume that Professor Z’s animals respond in the same way to vitamin C as those in Dr. A’s laboratory. This would have first to be proved experimentally, if any sort of comparison is to be

made of substances tested independently in the two laboratories. If, however, Professor Z could test the *same* lemon-juice as Dr. A, he could then compare his cabbage-leaves with it and thus make possible by indirect means a comparison of his cabbages with Dr. A's potatoes.

Lemon-juice does not keep very well: moreover, even though it may continue to look and taste the same, it loses its vitamin C slowly when exposed to air, especially if the temperature is somewhat high. Unless, therefore, the two laboratories are in close proximity and suited in other ways for carrying out contemporaneous tests with a common sample of some agreed "standard" preparation, they will have no common measure at all for comparing their two sets of results.

It was to meet this difficulty that the Health Organization of the League of Nations decided several years ago to set up "Standard Preparations" of certain vitamins, to define international units of these vitamins in terms of the Standard Preparations and to make those preparations available for distribution anywhere in the world where it was required to carry out biological estimations of the vitamins in question.

Five International Standard Preparations exist at the present moment, for the testing of vitamins A, B<sub>1</sub>, C, D and E. As we have confined ourselves in this chapter to the measurement of vitamin C, we will describe the standard preparation and unit of that vitamin in a little detail.

§ 34 Fortunately ascorbic acid itself is available as a pure, crystalline, stable white solid, and the International Standard Preparation of vitamin C is a particular sample of very pure ascorbic acid, of which a considerable stock is held, under conditions ensuring its stability, at the National Institute for Medical Research in London. Any worker who wants to carry out a vitamin-C assay on guinea-pigs can obtain from the Institute (or from his appropriate national distributing centre) a sample of this Standard Preparation, without any charge whatever. The International Unit of vitamin C

is *defined* as the anti-scorbutic activity contained in one-twentieth of a milligram of the sample of ascorbic acid that constitutes the International Standard Preparation.

§ 35 Let us, then, consider briefly the procedures of Dr. A and Professor Z, to both of whom the International Standard Preparation of vitamin C is available. Each worker will prepare two carefully matched groups of guinea-pigs—of whatever number of pairs of animals is necessary to get finally an equal growth response in test and control group; each will obtain a supply of the International Standard Preparation, and each will find by experiment how much of that preparation is necessary to give about half the maximum possible growth response, for reasons that we have already discussed.

Dr. A finds that  $\frac{1}{10}$  milligram of the Standard produces an average weekly gain in weight of 11 grams in a group of fifteen guinea-pigs receiving a scurvy-producing diet, and that an average gain of 12 grams is produced in another group of fourteen guinea-pigs by  $\frac{2}{3}$  of a gram of potatoes. Hence in anti-scorbutic activity  $\frac{1}{10}$  milligram of Standard is very slightly less effective than  $\frac{2}{3}$  gram of potatoes. But  $\frac{1}{10}$  milligram is two international units, by definition. Therefore,  $\frac{2}{3}$  gram of potatoes contains a little more than 2 units, and 1 gram of potatoes contains a little more than  $3\frac{1}{3}$  units, say  $3\frac{1}{2}$  units, of vitamin C.

Professor Z, on the other hand, finds that  $\frac{1}{15}$  milligram of the Standard is needed to give an average weekly weight-increase of 10 grams in fourteen animals, and his cabbage-leaves produce an average weekly gain of 9 grams when each animal of fifteen receives  $\frac{1}{12}$  of a gram daily. Hence, in anti-scorbutic activity  $\frac{1}{15}$  milligram of Standard is very slightly more effective than  $\frac{1}{12}$  gram of cabbage. Therefore, 1 milligram of Standard (20 international units) is slightly more effective than  $\frac{1}{12}$ —that is,  $1\frac{1}{4}$  grams of cabbage. Thus  $1\frac{1}{4}$  grams of cabbage contains slightly less than 20 units, so that 1 gram of cabbage contains about 15 International Units of vitamin C.

§ 36 It is very important to note the result of both workers' having compared test material with Standard Preparation. Suppose they had not done so, then



the only possibility for a comparison would have been to have compared the average weight increases of Dr. A's guinea-pigs receiving  $\frac{3}{8}$  gram of potatoes, and gaining 12 grams, with Professor Z's guinea-pigs receiving  $\frac{1}{12}$  gram of cabbage, and gaining 9 grams. We should then have argued that, as  $\frac{3}{8}$  gram of potatoes is distinctly richer in vitamin C than  $\frac{1}{12}$  gram of cabbage, 1 gram of potatoes is therefore superior to  $\frac{5}{36}$  gram of cabbage, and equal to, say,  $\frac{1}{6}$  gram cabbage, or less. So that the cabbage is six times as rich as the potatoes or even still more potent. Yet the evaluation in International Units actually showed a ratio of 15:3 $\frac{1}{2}$ —that is, a little over four to one. This is the more correct relation, for the use of the Standard Preparation has allowed the two investigators to correct for the *different* average responses of their animals to the *same* dose of ascorbic acid.

§37 We have dwelt at considerable length on this matter because it is one to which even to-day adequate attention is not always given. The most careful workers in vitamin assay are certainly those in this country, though many American investigators are equally punctilious. There are also in other countries honourable exceptions, but it is broadly true to say that there has been published a mass of material all over the world purporting to state the amounts of various vitamins in all sorts of foodstuffs, but based on experimental methods that almost completely ignore the essentials of biological assay described above.

It must be pointed out, however, that the criterion adopted of response to vitamin treatment for purposes of measurement need by no means always be growth—that is, gain in weight. For vitamin-D assay, the biochemist uses the extent of bone calcification; for vitamin-B<sub>1</sub> assay he may use the increased heart-beat of rats on a vitamin B<sub>1</sub>-free diet when B<sub>1</sub> is added to it, or even the effect on the rate of increase of fermenting yeast-cells; in vitamin-C assay itself he may use the effect on tooth structure. As long as he has some kind of scale by means of which he may attribute a numerical value to the response of each individual animal in a group, then he can

average the responses of all the animals in the group and express numerically the average response given by a measured dose of the Standard Preparation. *Unless* he can do this, he cannot carry out a vitamin assay; if he has *not* done this, he has not carried out a vitamin assay.

That is why we had not for some time any means of expressing, for example, the amount of vitamin E in a natural product or a medicinal preparation. There was no Standard Preparation, international or otherwise, and there could, therefore, be no unit and no assay. The best that could be done was, by experiments on carefully matched groups of animals, to express in terms of each other the vitamin-E contents of two contemporaneously tested sources. But that is *not* an assay, and affords no basis for comparison with another substance tested on other animals in different places or at other times. The absence of the yard-stick—the Standard Preparation—made it impossible in any way to allow for variations in response between the animals used in comparing the first two substances and those used for testing the third. This state of affairs was corrected, as soon as a sufficient number of investigators were interested in the problem, by the establishment in 1940 of an International Standard Preparation of vitamin E.

## CHAPTER XVI

### IDENTIFYING VITAMINS

§ 38 NEAR the beginning of this book reference has already been made to the use of cod-liver oil for curing rickets; as we have there pointed out, it would be more accurate to speak of the ability of some samples of cod-liver oil to produce some cure in some cases of rickets! In the light of the knowledge to be discussed in this chapter and later, we can say with confidence that many of the samples of cod-liver oil used in past centuries must have been completely devoid of any anti-rachitic activity. Even to-day samples of cod-liver oil produced under careful and up-to-date conditions may contain under 50 units per gram, while a very active cod-liver oil may contain as much as 300 units. Moreover, there are other fish-liver oils many times as rich, and values as high as 50,000 units per gram have been recorded.

Nevertheless, the association of anti-rachitic properties with cod-liver oil became firmly established, especially in the Scandinavian countries, and the variable responses of different cases to treatments apparently identical were just put on one side as unexplained mysteries. They are, indeed, still partly unexplained, but to-day we do at least know what are some of the causes of variation.

§ 39 At the beginning of the present century several discoveries were made that proved of the greatest importance for the ultimate isolation and identification of vitamin D. First, it was found that rickets could be cured not only by sunlight, but also by the rays from a quartz mercury-vapour lamp, without any dietary or medicinal treatment whatever. The effect of sunlight was used to explain a number of previously known facts—the *relative* frequency of rickets in sunny and in cloudy climates, in town and in country conditions, and the fact that, according to certain schools of thought, exer-

cise could cure rickets. Clouds and smoke apparently cut out from sunlight the rays really effective in curing or preventing rickets; exercise, whether of children or experimental animals, was frequently taken in bright sunlight.

How it was that a non-dietary treatment, such as irradiation with ultra-violet "light" from sun or lamp, could have the same effect as the oral administration of a fish-liver oil remained for some time completely baffling. The key to the mystery was found almost simultaneously by two investigators, but in the meantime another advance of great value had been made, mainly in the laboratories of United States scientists.

§ 40 As soon as the Swedish scientists, Holst and Frölich, had succeeded in producing experimental scurvy in guinea-pigs, which they did in 1907, there was quite a craze for producing other experimental deficiency diseases in suitable animals. Avian polyneuritis in pigeons was the laboratory equivalent of human beri-beri, and a similar condition, though of more complex origin, was produced in rats.

The production of experimental rickets in dogs was a fairly simple matter, but there were many objections, apart from purely sentimental and humane ones, to the use of dogs for this work. Fortunately, therefore, for dog-loving scientists (as they almost all are), in laboratories at Johns Hopkins and Columbia Universities, at Baltimore and New York respectively, more or less simultaneously and quite independently, a way was found to produce experimental rickets in rats.

The conditions for its production were, however, not identical with those apparently concerned in human rickets. Rats can do fairly well without any vitamin D, *provided* their diet is adequate in calcium and phosphorus. Such a vitamin-D-free diet does not, in fact, allow rats to develop quite normal bones, but it certainly does not give them rickets. If, however, the phosphorus of the diet is brought down to a very low level, without any corresponding lowering of the dietary calcium, then—provided vitamin D is absent from the diet—the rats

develop a condition of experimental rickets almost exactly like that found in the rachitic human infant.

§ 41 Like human rickets, this condition can be cured and prevented by cod-liver oil administration *or* by irradiation with ultra-violet rays from sun or lamp; like human rickets, it is accompanied by a marked lowering of the blood phosphorus, and sometimes by a distinct fall in blood calcium. As in human rickets, its prolongation leads to the development of wide cartilaginous zones at the growing points of bones, where normally lime salts—especially calcium phosphate and carbonate—would be deposited; this failure in calcification produces, as in human rickets, the characteristic softening, swelling, malformation and finally fracture of the bones—especially of the long bones of legs and arms.

Experimental rickets in rats can also be prevented and cured by restoring to the diet its deficient phosphorus—a procedure probably without effect on human rickets, which can apparently develop on a diet adequate in its calcium and phosphorus content, but lacking in vitamin D. In other words, more technical but terser, experimental rickets in rats is similar in pathology to clinical rickets in man, but it may well be different in etiology.

§ 42 This etiological difference between experimental rat rickets and clinical human rickets has certain important practical consequences. It means that we must argue only with caution from the nature, causes and cure of the one to those of the other; we can *use* rat rickets as a means of detecting and measuring vitamin D, for it can be cured by vitamin D, just like human rickets. But we must be careful not to be led astray by the pathological resemblances into overlooking the etiological differences.

Experimental rickets in rats is, as we have seen, due essentially to a shortage of phosphorus in the food, as a result of which there is a shortage in the blood-stream, which ultimately expresses itself in abnormalities of bone formation. Rickets in the human infant—and its adult analogue, osteomalacia—is due rather to shortage of vitamin D itself, whereby the calcium *and* phosphorus

in the food, however abundant, are not taken into the blood-stream, again with disastrous effects on the bones. The causes are different, but the results are the same.

Owing to this difference, rat rickets can be cured without adding any vitamin D at all to the rachitogenic—rickets-producing—diet of the rat. The direct addition of phosphate will produce the same result as the administration of vitamin D: it will cure or prevent the experimental rickets of rats. So will ammonium chloride, because its acidic nature enables it to free phosphoric acid from combination in the food and tissues and so to act like phosphate added to the diet. Conversely, the presence in the diet of unavailable, because unassimilable, phosphorus tends to produce rickets in rats; the phosphorus in certain cereals—especially oatmeal—is present in this form. These considerations do not appear to apply to man. Phosphates and ammonium chloride are ineffective in curing or preventing rickets in the human patient; oatmeal will not produce it, for the presence or absence of vitamin D is here the determining factor.

§ 43 It is frequently stated that breast-fed babies do not develop rickets, and some peculiar, unexplained virtue of human milk for human infants is generally implied—or indeed specifically claimed—as the reason. The claim, in any event, appears to be by no means a complete statement of the facts. It is legitimate to say that, in general, breast-fed infants seem less prone to rickets than those fed cow's milk, whether liquid or reconstituted from condensed or dried milk. But the difference is one of degree, not of kind. Infants brought up on cow's milk supplemented with vitamin D do not develop rickets save in the very rarest of instances, and they then belong to a very small group of cases suffering from "vitamin-D-resistant rickets," the cause of which is unknown; it can almost always be cured by exceptionally large doses of vitamin D. On the other hand, breast-fed infants of very poor mothers living in smoke-screened urban areas have frequently been diagnosed as rachitic to a greater or less degree. The most likely explanation of the available facts seems to be that breast-feeding is

bound to be confined to the healthier—and therefore on the whole the better-nourished—mothers in the community, so that one would expect healthier infants and a smaller incidence of rickets, as well as of other deficiency diseases. It is, moreover, known that the amount of vitamin D in the milk of any mammal is in any event largely determined by the mother's supplies, whether in the food or as the result of irradiation, though there appears to be an upper limit to this passage of vitamin D from the maternal blood-stream into the mammary glands and the milk. A similar "mammary barrier" has been demonstrated for the other fat-soluble vitamins, A, E and K.

Anyhow, the ease with which the newly-weaned rat can be given rickets by withdrawing vitamin D from its diet and reducing the intake of assimilable phosphorus (that is, of mineral phosphates) makes it invaluable as a means of testing for the presence of vitamin D. The investigator can control the amount of phosphorus in the rat's diet and make it almost as low as he likes; if, in spite of this, a product is given to the rachitic rat and cures its rickets, then that product *must* contain vitamin D, provided, of course, that the product is free from available phosphorus, which would have the same effect. That is why the discovery of how to produce rat rickets was of epoch-making importance.

It soon led to very rapid advances. First, as has already been stated, it made it possible to confirm the similar effects of ultra-violet light and cod-liver oil. Next, it led to the distinction between the vitamin A and the anti-rachitic vitamin of cod-liver oil, already discussed in §§ 11, 12, 13 and 14 of this Section. It soon became clear that, except for small amounts in egg-yolk and summer milk and butter, vitamin D occurred only in fish-liver oils. It was obvious that most people must be relying on solar irradiation for their protection from rickets or osteomalacia—a precarious protection in smoke-dimmed industrial cities. Rickets, even if only in its milder forms, is still to-day not completely eliminated among the child populations of our manufacturing towns.

§ 44 The next advance came from an almost simultaneous discovery by two more American scientists, Steenbock in Wisconsin and Hess in New York. Both of these workers found that rachitogenic diets would lose their ability to produce rickets if they themselves were submitted to the intense ultra-violet radiation of the mercury arc. This discovery opened the road that led directly to our present knowledge of the anti-rachitic vitamins and incidentally to increased knowledge over the much wider field of sterol chemistry in general.

For now the hunt was up. Immediate attempts were made to find out if any one particular ingredient of the food was responsible for the effect. It was. It was the fatty part of the food that developed under the influence of ultra-violet radiation the same anti-rachitic properties as had hitherto been found mainly in fish-liver oils. It did not seem to matter much what fat or oil was concerned—the commoner vegetable oils like olive oil and pea-nut oil, or the traces of fat present in such cereals as maize and wheat; all of them became anti-rachitic when irradiated. Moreover, the rat's skin—even if the rat had previously been given rickets—developed the same anti-rachitic properties. Further, an oil activated by irradiation could cure the experimental rickets of rats if it were rubbed into its skin! Here at last was a reconciliation between the disputant schools—those who said ultra-violet light cured rickets and those who said that some undiscovered constituent of fish-liver oil did so. Both were right. For the fat of the rat's skin—and presumably that of human beings also—becomes anti-rachitic on irradiation and thus renders the animal independent of external sources of vitamin D.

§ 45 The inquisitive minds of chemists did not, naturally, let matters rest here. The activatable portion of food—*pro-vitamin D*, as we now call it—had been traced down to its fatty constituent; in what part of the fat was it to be found? First the glycerides were absolved; the whole of the *pro-vitamin* was in the unsaponifiable matter. (See Chapter VI, especially §§ 26 *et seq.*) Next it was traced to the sterol portion of the unsaponifiable



matter. Cholesterol from animal fats and phytosterol from vegetable oils were both found to be rendered very strongly anti-rachitic by irradiation. For some time people thought that these sterols themselves might well be the pro-vitamins they were looking for. Some samples of cholesterol could be given an anti-rachitic activity—for human infants, as well as for rats—one thousand times that of a good cod-liver oil, so that this belief was by no means unreasonable. But the pure anti-rachitic vitamin was to turn out to be nearly a thousand times more active again!

After much laborious research it was found possible to purify cholesterol even further than had so far been done and to obtain samples that could *not* be made anti-rachitic on irradiation. Moreover, one of the ways of doing this was simply to irradiate cholesterol and to separate from it a small oily fraction—about one two-hundredth part of the cholesterol or less—which was proportionately more anti-rachitic than the cholesterol itself irradiated before the separation. When the unchanged cholesterol was recovered, ultra-violet light would no longer make it anti-rachitic.

§ 46 It therefore became certain that cholesterol itself could not possibly be pro-vitamin D. But there were some very interesting clues to suggest what was. First, the method of freeing cholesterol from its accompanying traces of pro-vitamin (apart from the procedure of *destroying* the pro-vitamin by actual irradiation) was such as to suggest that the pro-vitamin would be a substance more easily reduced than cholesterol. Secondly, its leech-like adherence to cholesterol was an indication, if nothing more, that it might be chemically allied to the sterols, if not an already known sterol.

The third clue was perhaps the most important. It was known with considerable accuracy which ultra-violet radiations were most effective in making cholesterol anti-rachitic; they were the ones with a wave-length somewhere near 300 m $\mu$  (millimicrons—that is, millionths of a millimetre). Now physical chemists had established a very important principle—important to have established experimentally, even though it sounded rather obvious

once it had been proclaimed! It was this: any particular wave-length of the spectrum, visible or invisible, that is capable of causing a particular chemical compound to undergo chemical change *must* be absorbed by that compound. From this it followed that the compound must show in its spectrum an *absorption band* exactly at the wave-length of the chemically effective radiation. Consequently activatable cholesterol *must* show an absorption band near 300  $m\mu$ . Spectroscopic examination not only revealed the presence of this band, but also showed that the band gradually disappeared during irradiation, and that the intensity of the band was roughly proportional to the degree of anti-rachitic activity that could be conferred on any sample of cholesterol. Purified cholesterol that remained unaffected by irradiation showed no absorption band at all at 300  $m\mu$ .

§ 47 All these clues pointed to certain tentative conclusions. Pro-vitamin D, it was foretold, would turn out to be a sterol, or something very closely related to a sterol, of the more easily reducible kind; it should have an extremely intense absorption band near 300  $m\mu$ , since the traces of it in activatable cholesterol (certainly under 1 per cent.) gave to that substance an easily observed band.

It is to Windaus of Göttingen that must go the credit of putting one and one and one together and making three. Many years ago Tanret, a French chemist, had separated a new sterol from the oil of ergot of rye. This he had called ergosterol. Windaus found that ergosterol absorbed very strongly indeed between 260 and 294  $m\mu$ , showing no less than four separate absorption bands in this region. When ergosterol was irradiated with ultra-violet light consisting mainly of radiations within those limits, several changes took place. The original bands disappeared, and were replaced by a single band near 270  $m\mu$ . This also disappeared as irradiation went on. At an appropriate moment during the existence of the 270  $m\mu$  band, the irradiated ergosterol was found to have a staggeringly high anti-rachitic activity, some 20,000–50,000 times that of cod-liver oil, and some 100 times that of the most active irradiated cholesterol.

It certainly seemed as if the matter were now settled; following upon a masterly alternation of experimental work and logical deduction, we were now in possession of an anti-rachitic substance immeasurably more potent than anything previously known. Clinical trial soon established its beneficial action on the rachitic infant and its ability to prevent rickets when no other form of treatment, dietary or environmental, was used. Had we, at last, got possession by indirect means of the pure vitamin that existed in cod-liver oil only as a relatively insignificant contaminant?

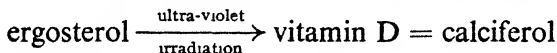
§ 48 For a time there was a lull on this vitamin front. Irradiated ergosterol was a waxy-looking, semi-solid mass, from which unchanged ergosterol could be fairly easily removed. This operation left over a yellowish-brown resin of which the anti-rachitic activity was about twice that of the crude irradiated material before removal of unchanged ergosterol.

Windaus and his colleagues and also an independent group of workers at the National Institute of Medical Research in Hampstead set themselves the task of separating this resin into various parts, in the belief that it was a mixture of several substances and in the hope that one of these substances would turn out to be a solid, crystalline pure anti-rachitic vitamin. They were right, and both teams succeeded, almost simultaneously, though by different routes, in isolating from irradiated ergosterol the substance now almost universally called calciferol (as suggested by the English team), but still given by some workers Windaus's name "vitamin D<sub>e</sub>" (vitamin D<sub>1</sub> was a less pure crystalline substance, a mixture of calciferol with something else, separated by Windaus a few weeks earlier; the subscripts 1 and 2 are, however, generally preserved for historical reasons).

It is, by the way, extremely gratifying to note that the rivalry between these two groups, culminating in their separate isolation of calciferol, was concluded in an atmosphere of cordial co-operation; they not only communicated their methods to one another, but they also exchanged specimens and confirmed the identity of each

other's crystalline by-products. It was a fitting end to a magnificent chapter in international biochemistry. But it was not the end of the whole story.

§ 49 It must never be forgotten that at every stage in this long chain of research the cumbersome, inaccurate, lengthy and expensive procedure of animal tests had to be used to check the work of the chemists. Moreover, there were no Standard Preparations available in those early days; the only way of finding out anything quantitative was simply to compare one product with another. Besides, the fundamental principles of bio-assay, as discussed in the last chapter, were very little appreciated then, and workers often deceived themselves by comparing results obtained in different laboratories, with different stocks of animals and under considerably different conditions of test. We have only quite recently come to realize how much of the quantitative data reported at that time must be discounted and regarded as, indeed, little more than qualitative. In spite of this handicap—an extremely serious one, too—the work went on, because it soon became evident that the relationship



simply would not cover all the facts.

§ 50 Crude solutions of irradiated ergosterol and, later, calciferol itself were very carefully assayed as soon as the International Standard Preparation came into existence. The former showed activities varying from a relatively low figure up to 5,000,000 units per gram (or 10,000,000 units, allowing for the unchanged ergosterol present); the latter was found to have a constant value of 40,000,000 international units per gram. Subsequent work has entirely confirmed this figure, which is now universally accepted as the true anti-rachitic activity of calciferol.

It will be remembered that this statement has a simple and definite meaning. It means that a sample of *pure* calciferol, free from all non-anti-rachitic contaminants, will have an effect on the experimental rickets of rats

exactly 40,000 times that of the International Standard Preparation, which contains by definition 1,000 international units per gram.

The non-calciferol portions of irradiated ergosterol were found to be entirely devoid of anti-rachitic activity. One of them has been obtained in a pure crystalline state, and is as physiologically inert as non-irradiated ergosterol itself. Others have been found to have a marked toxic action on rats and mice when administered in very high doses, and this is also true of calciferol itself. But the other toxic substances in crude irradiated ergosterol (called tachysterol and toxisterol) are entirely without the intense anti-rachitic action of calciferol. It follows that, for a given intensity of anti-rachitic activity, calciferol will be several times less toxic to rats and mice than irradiated ergosterol.

§ 51 Calciferol was slowly becoming accepted as the only known anti-rachitic substance, in spite of the scepticism of certain clinicians. A good deal of this scepticism must be put down to that subconscious primitive Nature-worship that still seems to persist in many laymen and some doctors and causes them to maintain with imperturbable obscurantism that "Nature knows best," and presumably still to prefer that their aspirin should be made from "natural" oil of winter-green instead of from "synthetic" coal-tar salicylic acid! But evidence of a very different order, based on carefully controlled experiments, became available when Massengale and Nussmeier threw a most unwelcome spanner into the works.

Their discovery depended on the already known fact that chickens need anti-rachitic treatment; otherwise they develop "leg-weakness" and die. Examination of the bones of chickens lost in this manner shows their condition to be in many respects similar to the bones of rachitic rats or human infants. It had been known for many years that cod-liver oil is very effective as a prevention of this condition in chickens. The two American scientists found, however, that irradiated ergosterol was quite astonishingly ineffective. Their work has been

repeated a number of times—always with results that were qualitatively the same, though there was a good deal of quantitative difference between the results obtained in different laboratories. We now know that differing experimental conditions were largely responsible for these quantitative differences.

Let us put the matter, nevertheless, in numerical form. Suppose we assay a sample of cod-liver oil, using the accepted method—that is comparing its effect on rats with that of the International Standard Preparation—and suppose we find it comes out at just 100 international units to the gram. We might then find that 2 grams a day were amply sufficient to protect a newly-hatched chick from leg-weakness; that means that the prophylactic dose of vitamin D for this chicken is 200 units per day—or less. Now we will make up a solution of calciferol in oil so that every gram contains 100 International Units; this figure is, of course, based upon assays of calciferol carried out on rats in the same way as the cod-liver oil assays. (The solution, since calciferol contains 40,000,000 units per gram, will obviously contain  $\frac{1}{400,000}$  gram of calciferol in every gram.) Now, if calciferol is the same as the vitamin D in cod-liver oil, this calciferol solution should be *exactly* as effective as the cod-liver oil containing 100 units per gram; 2 grams of the calciferol solution should completely prevent any signs of chicken-rickets or “leg-weakness.” In fact not twice or ten times the amount will do this; as much as fifty times may be required, or even more. We cannot, of course, give a chicken 50 grams of the particular oil solution; we have to increase the amount of calciferol in solution, and feed stronger and stronger solutions until we reach one that is effective. In fact the most effective treatment with calciferol never equals the most effective treatment with cod-liver oil in chickens, as judged by the amount of calcium and phosphorus laid down in their growing bones.

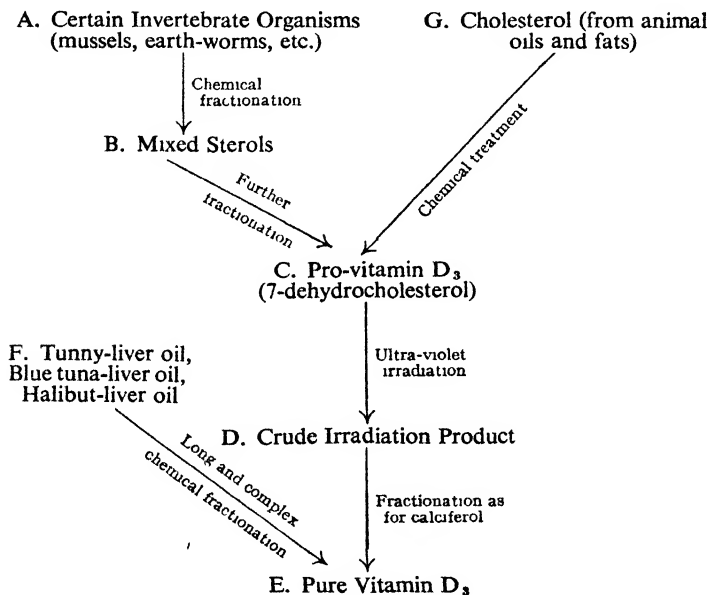
§ 52 Attempts were made to show that this curious ineffectiveness of calciferol was apparent and not real; that the chicken needed the vitamin A of the cod-

liver oil, as well as its vitamin D, for bone formation and freedom from leg-weakness. The attempts failed. *No* other explanation than one fits the facts; the vitamin D in cod-liver oil is *not* calciferol and chickens have a curious insensitivity to calciferol—an insensitivity not shared by the rat. Such a deduction from the experimental facts leads at once to the very pertinent question, What about the human species? Do infants behave, *vis-à-vis* their anti-rachitic vitamin, more like chickens than like rats? Must we abandon the use of calciferol, with its fallacious appearance of 40,000,000 units to the gram, and return to the mild but effective, if unpleasant, oil from the cod's liver?

The answer is, "No, no—a million units, no!" Man is, fortunately, exactly, or almost exactly, like the rat and quite unlike the chicken. We now know that there exist in nature at least two, and probably more than two, forms of vitamin D. It is nonsense to distinguish between cod-liver oil vitamin D and calciferol as natural and artificial, because the latter exists in nature—in certain moulds, in some samples of ergot of rye and in the husk of the cocoa-bean—while the former has been made by methods no less, and no more, "artificial" than those used to prepare calciferol. If it were necessary to find adjectives for distinguishing between the two anti-rachitic vitamins, then it would be legitimate to talk of "animal" and "vegetable" vitamin D.

§ 53 It is now possible to prepare crystalline vitamin D<sub>3</sub>, and there is good reason to believe that it is identical with the anti-rachitic vitamin of cod-liver oil. It is, like calciferol, a product of irradiation, but its pro-vitamin is not ergosterol. Indeed, we now believe that the pro-vitamin present in the cholesterol of the early workers never was ergosterol at all, but the pro-vitamin of vitamin D<sub>3</sub>. It is a substance very closely related *both* to cholesterol *and* to ergosterol. Apparently its relation to cholesterol is what causes the corresponding vitamin D<sub>3</sub> to be preferred by chickens to calciferol, but its relationship to ergosterol is what causes it to be susceptible of anti-rachitic activation by ultra-violet light.

The diagram gives an indication of how vitamin  $D_3$  has been separated or "synthesized." The vitamin has been separated in pure crystalline form from certain fish-liver oils (route F E); it has also been prepared by irradiation of its pro-vitamin, 7-dehydrocholesterol (route C D E). The pro-vitamin itself has been isolated from natural sources (route A B C) and has also been chemically prepared from cholesterol (route G C).



§ 54 Pure vitamin  $D_3$  has now been made in sufficient amounts for extensive laboratory and clinical trials. Sufficient is known for it to be stated:—

1. That its anti-rachitic activity, assayed biologically on rats, is about the same as that of calciferol—namely, 40,000,000 international units per gram.
2. That its effect on chickens is the same as that



of cod-liver oil, if both are administered on the basis of the same number of units; it follows from 1 above that this means in equal weights.

3. That its method of laboratory preparation by irradiation of its pro-vitamin is by a route exactly parallel to the preparation of calciferol.

4. That in clinical use for protection from rickets, and the prevention of increase in severity of existing rachitic symptoms, the most careful investigation yet conducted has failed to show any detectable difference between sources of calciferol and sources of vitamin D<sub>3</sub>.

5. That reports of the superiority of vitamin D<sub>3</sub> over calciferol in the cure of marked or severe rickets are based on only a small number of patients, and in any event do not indicate a difference in efficiency of more than 2 units to 1, if as much.

6. That most comparisons of calciferol and vitamin D<sub>3</sub> for prevention or cure of human rickets have failed to establish any significant difference.

7. That in the treatment of another, and rare, human illness, due to insufficient secretion of the hormone from the parathyroid gland, very large doses of vitamin D given periodically and continuously have proved highly satisfactory. Little if any difference has been found between the efficacies of calciferol and vitamin D<sub>3</sub> in treating this condition.

§ 55 We can therefore safely conclude that calciferol will continue, at any rate for some time, to be the anti-rachitic agent of choice, whether for use in medicinal preparations or for additions, under careful control, to certain foods, especially margarine, intended for human consumption. Its efficiency as a prophylactic against rickets has been proved up to the hilt; in spite of the mumblings of sceptics and hypercritics, the ill effects of administering even enormous doses of this "artificial" vitamin to human beings still await the slightest convincing demonstration, and in the small doses used for

activating foodstuffs it is no more toxic than common salt. It is cheap and available in small or large quantities to reputable food manufacturers. There is not the slightest scientific reason, taking all these facts into consideration, why infantile rickets should not in three years be entirely eliminated from the world. Indeed the daily prophylactic requirements of the whole child population in Great Britain, if supplied as calciferol at the lowest possible manufacturing price, would be about £250—that is, assuming a child population of 5,000,000 and allowing every child a *generous* prophylactic dose. The total annual cost would therefore be well under £100,000, which I can leave to the reader to evaluate in terms of bombers and tanks. The expenditure would completely and finally eradicate rickets from these islands, though of course it would have to include the cost of suitable distributive and administrative measures, say £500,000 or even £1,000,000 annually.

Scientists have, without any doubt, tracked down and identified this vitamin. Calciferol is there, ready and available; rickets has still to be eliminated. Presumably the cost is too high.

Other substances with slight or marked anti-rachitic action are also known. They are all, however, the products of special laboratory experiments and have not so far been found in natural products; they are, therefore, of no immediate practical interest from the standpoint of nutrition.

§ 56 By equally arduous, though very different, routes other vitamins have been obtained as pure synthetic substances. There are ascorbic acid (vitamin C), aneurine chloride (vitamin B<sub>1</sub>), riboflavine, nicotinic acid, tocopherol (vitamin E) and others, already mentioned in Chapter XIV, §§ 20 *et seq.* (pp. 89 to 91). The constitution of vitamin A is also known; it has been separated, but not yet synthesized as a single pure substance (though that might be achieved at any moment). For those who are interested in the chemical nature of this very varied group of dietary essentials, our knowledge of their composition has been summarized in Table III.

TABLE III

*The Chemical Elements in the Vitamins and Pro-Vitamins*

Designation	Name	Empirical Formula
Vitamin A	—	$C_{30}H_{48}O$
Pro-vitamin A	Carotene	$C_{40}H_{56}$
Vitamin B <sub>1</sub>	Aneurine, thiamin	$C_{12}H_{17}N_4SClO$
"Vitamin B <sub>2</sub> Complex"	Riboflavine	$C_{17}H_{20}N_4O_6$
	Nicotinic Acid	$C_6H_5NO_2$
	Pyridoxin	$C_8H_{11}NO_3$
	Pantothenic Acid	$C_9H_{17}NO_5$
	Biotin	$C_{10}H_{16}N_2SO_3$
	Folic Acid	$C_{19}H_{19}N_7O_6$
	[Inositol]	$C_6H_{12}O_6$
	[Choline]	$C_5H_{15}NO_2$
	[ <i>para</i> -Aminobenzoic Acid]	$C_7H_7NO_2$
Vitamin C	Ascorbic Acid	
Vitamin D <sub>2</sub>	Calciferol	} $C_{28}H_{44}O$
Pro-vitamin D <sub>2</sub>	Ergosterol	
Vitamin D <sub>3</sub>	—	} $C_{27}H_{41}O$
Pro-vitamin D <sub>3</sub>	7-Dehydrocholesterol	
Vitamin E	$\alpha$ -Tocopherol	$C_{29}H_{49}O_2$
Vitamin P	Hesperidin, etc.	$C_{28}H_{34}O_{15}$ , others uncharacterized
Vitamin K <sub>1</sub>	—	$C_{31}H_{46}O_2$
Vitamin K <sub>2</sub>	—	$C_{41}H_{56}O_2$
"Synthetic vitamin K"	Methylnaphthoquinone (Menaphthone, Menadione)	} $C_{11}H_8O_2$

## CHAPTER XVII

### DEFICIENCY DISEASES

§ 57 THE term deficiency disease is generally used in a restricted sense, to describe a condition due to shortage in the body of some particular dietary constituent. Indeed, it is sometimes still further restricted to vitamin-deficiency diseases. We have already seen that nutritional anæmia is due to a shortage of iron, and of red-blood cells, in the blood, following the consumption of a diet inadequate in its iron content. The "coastal sickness" of sheep is a cobalt-deficiency disease; in South Africa cattle may suffer from shortage of phosphorus if they pasture upon soils that contain too little of the element. This aphosphorosis has been responsible for immense losses.

In fact any sick condition due to the absence or shortage of dietary constituents may rightly be termed a deficiency disease. The most complex and complete deficiency disease is starvation; like other deficiency diseases, it ends fatally, unless curative measures are taken.

In this chapter we shall deal very briefly with the clear-cut vitamin-deficiency diseases. It must, however, never be forgotten that partial vitamin deficiency, and especially partial deficiencies of more than one vitamin, are responsible for much of the general ill-defined bad health or "malnutrition" to which reference is made. There is, of course, no hard-and-fast line, except in so far as a line is to be drawn between illness and ill-health; the division is simply one of convenience. But its arbitrary nature must always be remembered, and we must not be misled into making absolute what is a purely relative distinction.

§ 58 Beri-beri, due to deficiency of vitamin B<sub>1</sub>, aneurine chloride, is associated with various symptoms: shortness of breath, paralysis, œdema (dropsical swellings) and numbness, with loss of sensation, especially in the

hands and feet. It is prevalent among poor rice-eating peoples, especially when the husks of the rice, which are known as polishings and are rich in aneurine chloride, have been removed in milling. In the experimental animal similar symptoms are observed, and the avian polyneuritis of the B<sub>1</sub>-deficient pigeon has been much used in laboratory work on the vitamin.

§ 59 Scurvy is due to the absence of ascorbic acid (vitamin C) from the diet. It is associated with lethargy, pains in the joints, sallowness of complexion, subcutaneous hæmorrhages, sponginess of the gums, looseness of the teeth, fragility of the bones and often œdema. It has been called the "mariners' disease," for it used to be a terrible scourge on long voyages, when seamen were for weeks unable to obtain fresh fruit, vegetables or meat. We have already considered the experimental scurvy of guinea-pigs, in Chapter XV. Some animals—the rat, for example—cannot be given scurvy, and it has been shown that the rat has the power, withheld from man and some other mammals, of synthesizing its own ascorbic acid.

We have to-day, moreover, to recognize that scurvy may sometimes possibly be a multiple deficiency disease, shortage of ascorbic acid being complicated by shortage of the substance or substances considered to have vitamin P activity (see pp. 88 and 89).

§ 60 Rickets in human children, and osteomalacia in adults, due to a deficiency of vitamin D—either because it is absent from the ingested food and medicines or because the individual is not receiving effective irradiation with the appropriate ultra-violet radiations or for both reasons. Though, unlike beri-beri and scurvy, it is seldom directly fatal, the effect of rickets on the individual is extremely serious and may well last into adult life. Rachitic female infants may suffer from deformations of the pelvis that are later sources of grave danger to themselves and their babies during child-birth.

§ 61 Dangerous shortage of vitamin A is fortunately not common, but it was experienced rather widely during the 1914–18 war in places where dairy produce and green

vegetables (or carrots) had become inaccessible. In its extreme form it is characterized by night-blindness; we now know that vitamin A is necessary to form one of the sensitive pigments in the retina. During the last few years evidence has accumulated to show that milder deficiency of vitamin A (or carotene) may cause impaired ability to see in dim light, more specifically a raising of the dark-adaptation threshold—that is, the lowest intensity of light at which objects can be distinguished—and a prolonged time of adaptation to low light intensities. It must, however, be emphasized, first, that the measurement of dark adaptation calls for great skill and critical judgment from the experimenter: such tests should always be carried out by or in collaboration with a competent ophthalmologist, and even he needs practice to become perfect at it: secondly, that by no means all cases of impaired dark-adaptation are due to vitamin A deficiency or to that deficiency alone. There appear to be congenital forms, and there is also some evidence that other vitamins (*e.g.*, ascorbic acid or riboflavine) have a part to play in the full effectiveness of the visual mechanism concerned. Deficiency of vitamin A also involves degenerative changes in the mucous membranes of the respiratory and alimentary tracts and of the urogenital system. These tracts have normally a marked ability to resist the invasion of bacteria; in vitamin-A deficiency this lessens to vanishing-point, and the victim is likely to succumb to the consequent secondary infections.

§ 62 Pellagra is endemic in certain districts where the population exists largely on maize; parts of the United States, Italy and certain Balkan countries have been heavily afflicted with the disease. Ulceration of the mouth and reddening of the skin, especially after exposure to sunlight, or other irritation, mark the earlier stages of the disease, which ends in death, with enteritis, general dermatitis and degenerative changes in spinal cord and nerves. It is curable (in the early stages) by yeast and yeast extract and by other foods (liver, cereal germ) rich in nicotinic acid, but it is possible, as already pointed out (p. 87), that other members of the

vitamin B<sub>2</sub> complex—riboflavine, for example—may be involved in this curative effect.

Vitamin K deficiency has been extensively studied in two very different sets of conditions. In obstructive jaundice, where a tumour may impede the flow of bile into the intestine, there is almost always a loss of clotting-power in the blood. This may prove a dangerous hazard if the patient has to have an operation. It is now almost invariably prevented by administering vitamin K pre-operatively. Again, new-born infants have been found, even when they and their mothers are well nourished and perfectly healthy, to have a similar low clotting-power of the blood. In most instances this is of little import, as the "abnormality" disappears in a few days after the baby's birth. But if delivery is difficult, or if the newly-born infant is likely to be damaged for any other reason, this loss of clotting power can be very serious. It appears possible to confer on the newly-born the blood-clotting power of the adult by injecting vitamin K either into the baby or into the mother shortly before delivery. In both the types of case here described the blood abnormality is due to its low content of a highly specific protein—called *pro-thrombin*—which is normally formed in the liver and is one of the three blood constituents essential for normal clotting. (Incidentally, the condition here described is quite distinct from the congenital disease *hæmophilia*; this is caused by shortage of one of the other two essential constituents.) Fortunately, the chemical pathologist has available very sensitive (though rather difficult) tests for measuring the amount of prothrombin in the blood: when vitamin K is not going to be administered as a routine, these tests can point at once to the need for it and can also show when the treatment has been effective. How vitamin K acts in stimulating the liver to form prothrombin is so far entirely unknown, but it always does so when the amount in the blood is low, except when the liver itself is seriously damaged. In what has been written above, the phrase "vitamin K" has been used for convenience: the simple synthetic compound, methyl-naphthoquinone (see pp. 89 and 90),

or one of several closely allied compounds, has entirely replaced vitamin K in clinical practice.

§ 63 Shortage of the other well-defined vitamins—riboflavine and vitamin E, for example—has not been so far considered the cause of any simple deficiency diseases. There are, however, other manifestations of vitamin shortage besides the frank deficiency diseases. The effects of inadequate vitamin supplies must be considered as part of the more general problem of inadequate diets.

Recent work has tended to put pernicious anæmia among the conditions that may be in part deficiency diseases. There is present in many foods a substance called folic acid, which is generally regarded as part of the vitamin B<sub>2</sub> complex and has been found essential to the life of several species of animals and many bacteria. This compound, which has been synthesized, may in adequate dosage have a remarkably beneficial effect on true Addisonian pernicious anæmia, as well as on certain tropical anæmias, like that of sprue. As folic acid is present in foods and is certainly different from the anti-anæmic factor of liver, the remission of pernicious anæmia gained from oral administration of folic acid would almost warrant including the disease under the general title of this chapter.



## SECTION V—DIET AND HUMAN HEALTH

### CHAPTER XVIII

#### THE NECESSITY FOR THE OPTIMAL

§ 1 THE main task of this book has been accomplished ; it is for the reader to judge how successfully. Its aim has been to put before him the kind of methods used in the laboratory study of nutritional problems and the kind of knowledge obtained thereby. To attempt the further task of showing how this knowledge bears on problems of human diet would require another book of at least the same length—and even then only the barest outlines could be given. Rather have I tried to put the reader in a position to apply some degree of scientific criticism to the many dietary proposals that are increasingly to be found both in the lay press and in the more technical journals: the importance to the democracies of an intelligent and well-founded public interest in nutrition, both during the war and afterwards, hardly needs emphasizing.

No scientific investigation can be properly evaluated except in relation to the circumstances that, directly or indirectly, were its origin and to the applications that may be made of its results. In order, therefore, to round off this outline it becomes necessary to say something about the bearing of nutritional research on the food and health of man. In so doing I shall perforce lay myself very much open to the charge of dogmatism and even bias, foreign to the objective spirit of scientific inquiry, as there will be little space for the saving clause and the qualifying parenthesis. In writing this concluding section, therefore, one hope will be very prominent in my mind—that the reader, when reading it, will himself apply that degree of scientific criticism to which I have tried to assist him.

§ 2 I propose, then, to lay down certain general propositions which, in my opinion, would be accepted by most of those who have worked on nutritional problems in the laboratory or in the wider field of applied dietetics, or in both. First, that the results of animal experiments are applicable, with due caution, to the problems of human nutrition and health. Secondly, that inadequate feeding, in quantity and quality of food, is responsible for a vast amount of ill-health and serious disease. Thirdly, and as a direct corollary of the second generalization, that improved dietary conditions would bring incalculable improvement to the health of vast numbers of people, in "civilized" and "primitive" communities alike. Fourthly, that such improvement could to some extent be brought about by educational methods, to both children and adults and to both rich and poor. Fifthly, that a far greater improvement would result from securing that all who serve the community by working are so remunerated as to make adequate expenditure on food possible for them all. Sixthly, that such a possibility, whether brought about by a reduction in retail prices or an increase in wage levels or both, would in fact lead to that improvement in diet necessary for a general improvement in health. In other words, food does constitute for the majority of people the first call on income, after the payment of essential overheads, like rent, heat, light and clothing; a large portion of any increase in real income to the poorer paid workers would generally be used for buying more and better food.

§ 3 During the last few years there has been much discussion of the essential dietary needs among populations in Western industrial countries. The literature on the subject is very large and fills many pages in many journals; yet, in spite of the importance of the subject, there is still some lack of agreement among the experts. Even a superficial examination, however, of their recommendations shows at once that there are certain basic causes for the disagreement.\* In the first place, very different methods have been used for assessing needs, and, in the second place, there has been no

uniformity in defining exactly the needs themselves. The dietary requirements of an individual will be largely determined by the state of health to which we desire that his diet shall raise him. Unless we define with some care the exact level of nutritional well-being at which we are aiming, it is obviously impossible to define what we mean by dietetic requirements. So it happens that proposals for adequate diets have varied, as they were bound to, according as the particular investigators have aimed at a minimum diet, a safe diet, a normal diet, a good diet and so on.

A rather alarming feature about official and semi-official investigations into this matter, and the reports that have followed from them, has been that they have not always been undertaken with the object of laying down an optimum diet. An optimum diet is the simplest to define and to make the object of a dietary scheme and is open to few of the subjective differences of interpretation that characterize some of the other dietary levels. An optimum diet can be defined as one that, for any individual or group of individuals, cannot be improved by increasing (or diminishing) any of its constituents. By this is not, of course, meant any single food, since most, if not all; dietary constituents may be obtained from a variety of foods. The diet must be considered as a whole; the sum total of each individual constituent must be such that it is not capable of quantitative improvement. The quantitative then passes into the qualitative; such a diet, in which each constituent is at its quantitative optimum, is also qualitatively optimal.

Now, it may be true that to define precisely an optimum diet for any one individual, let alone for a group, class or nation, may not be possible with great accuracy, but it is certainly easier to define such a diet in the light of present-day knowledge than to define one that shall be only "minimal" or "safe." We shall, moreover, do this with the greater certainty if we always err on the generous side. Let us, then, try to lay down, as accurately as may be, what amounts of each of the known essential

dietary constituents are necessary for maintaining, say, the British urban industrial worker in the best possible health that can be achieved by nutritional means alone. The fact that such a diet may still often be beyond the reach of the average, or even the best-paid, urban industrial worker, is not germane to the purely dietary aspect of the problem, however important it may be to the politician and—in his capacity as a private citizen—to the dietary expert himself. When a constructional firm is asked to tender for a railway bridge, it is asked to tender for a railway bridge that will do its job as well as possible and not for one that will be just “safe” or “minimal” within the financial ability of the customer to pay. It seems unfortunate that a different principle should be adopted in matters of human construction.

§ 4 One of the first things to remember about an optimum diet is that there is no need to bother about its fuel or calorie content. If there is enough money to pay for enough food to contain enough of all necessary food constituents, it is quite certain that the primary call of hunger, which is mainly an expression of the need for calories, will be easily satisfied. Even on a basis of protein alone, this is certain. We can take the protein requirements of an adult on an optimum diet as about 120 grams per day, of which at least half should consist of biologically first-class protein; the 120 grams will themselves supply some 420 Calories. We shall find it very difficult to make up an all-round mixed diet containing this amount of protein in less than one kilogram of food; even if 200 grams of this kilogram consist of water, we are left with about 700 grams of fat and carbohydrate or, to make very modest claims, 500 grams, after allowing a further 100 grams for mineral constituents, roughage and other items that do not contribute to the calorific intake. Even if the carbohydrate predominates, 500 grams of mixed carbohydrate and fat must furnish at least 2,000 Calories; if it includes any normal proportion of fat, it will furnish 2,500 Calories, which, with the protein already mentioned, will make up about 3,000 Calories altogether. Since these calculations have been based on very modest

assumptions, it is certain that this number of Calories will generally be exceeded by a good all-round diet satisfying other essential criteria and containing 100–120 grams of protein.

The optimum requirements for carbohydrate can, therefore, be ignored altogether, since they will follow inevitably from an optimum consumption of protein and other less obvious essentials. The experts differ somewhat as to the optimum proportion of fat to carbohydrate in a mixed diet; it is almost legitimate to state that, provided the requirements of fat-soluble vitamins are met, and provided there are no abnormal demands on the energy content of the diet by way of heavy manual labour, fat itself is hardly an essential dietary constituent at all. It is largely, then, a matter of taste how the balance between fat and carbohydrate is maintained for different individuals, and this taste will be partly conditioned by the primary physiological need for calories. A given weight of fat affords about two and a quarter times the calories given by the same weight of carbohydrate.

There is no need to discuss in detail the sources of protein, fat and carbohydrate, since they are to be found in all the ordinary staple foods. It is, however, worth recalling that an adequate intake of protein—that is to say, of the *essential* amino-acids, some half of the total number of amino-acids occurring in the human body—will be most easily secured from a diet containing about 50 per cent. of its protein as animal protein, derived from meat, fish, poultry or dairy produce; with less animal protein in a diet it is necessary to depend upon the “complementary” action of vegetable proteins for an adequate supply of all the necessary amino-acids.

§ 5 Just as it may be said with general truth, “Look after the proteins and the calories will take care of themselves,” so may it also be said, “Look after the calcium and iron and the rest of the minerals will look after themselves.” It is almost certain that small but essential amounts of copper, and possibly of other elements like manganese and cobalt, will accompany the iron in foodstuffs, if the iron is available in sufficient

quantity. For an optimal diet, the daily intake of iron should be 15 milligrams a day. The "offals" of the animal body—that is, the liver, heart, kidney and spleen—afford excellent sources of dietary iron; so do egg-yolk and certain vegetables, particularly spinach, as well as some fish, some nuts and, perhaps most important of all, whole wheat. White flour is a less good source of iron; the superiority of the whole grain as a source of iron is an argument that has been little exploited by the advocates of wholemeal bread. Two and a half pounds of wholemeal bread contain about 15 milligrams of assimilable iron. As has been pointed out earlier (p. 56), cooked meat is now also considered to be a satisfactory source of available iron.

The adult requirement for calcium in an optimum diet may be put at 1 gram per day at least, probably at 2 grams. This should be mainly obtainable from dairy products, though the abandonment of the habit of crunching bones, doubtless prevalent among our prehistoric ancestors, may perhaps be regretted by the punctilious nutritionist. However, there are other sources of calcium, such as the outer leaves of vegetables—found to be the preferential choice of the rabbit, in this respect a better dietitian than man, who prefers the "heart" of the vegetable—the pulses, egg-yolk and, though this is sometimes overlooked, certain processed fish, such as kippers, bloaters, sardines, pilchards, in which the small bones, being ubiquitous and considerably softened by the processing, not only can hardly be avoided, but can be swallowed with little or no loss of dignity!

Although, physiologically speaking, phosphorus is perhaps an even more important element than calcium, it is safe to say that an optimum intake of calcium will automatically secure an optimum intake of phosphorus. It is also true that magnesium, an essential dietary element, follows calcium very closely, so that an adequate calcium supply is a guarantee against magnesium deficiency.

§ 6 Having disposed of the major dietary constituents and the minerals, we find that the problem of the

vitamins has simplified itself very much. The use of wholemeal cereal or high extraction flour and green leaves, as the best sources of certain indispensable minerals, automatically secures an adequate intake of ascorbic acid (vitamin C), aneurine chloride (vitamin B<sub>1</sub>), nicotinic acid, carotene (pro-vitamin A) and vitamin E; on the other hand, abundant dairy produce, included as a source of calcium and animal protein, brings with it a corresponding intake of riboflavine and other members of the "vitamin-B<sub>2</sub> complex," with some vitamin A and, at any rate in summer, a certain amount of vitamin D.

A diet consisting largely of dairy produce, green vegetables, wholemeal or national bread and meat or fish, with additions of eggs and pulses, is pretty certain to be adequate in all essential dietary constituents, with the possible exception of vitamin D. Man has evolved in such a way as to make him independent of food for his vitamin-D requirements, since he has the ability to make this for himself in bright sunlight. If the conditions of our industrial civilization make his access to bright sunlight insufficient, then he must get his vitamin D in some other form, whether he makes use of the ingenuity of the organic chemist or the courtesy of the cod-fish, whose liver is always at his disposal. Some authorities think it likely that with optimal intakes of calcium and phosphorus, which implies also an optimal ratio of the two elements, the vitamin-D requirements of man may be materially less than they are when either of the elements is being taken in sub-optimal amounts. In any event, compensatory daily doses of vitamin D are so easy to obtain that there is not the least excuse, even with sub-optimal quantities of calcium and phosphorus in the diet, for the appearance of any manifestations of vitamin-D shortage in any member of a "civilized" community.

§ 7 An attempt has been made in this chapter to show that the essentials of an optimal diet are so well known to us, so simple and so easy of access that there is not the slightest justification for the existence of malnutrition or even of sub-optimal nutrition. Any

figures given here have been, admittedly, approximations; but so must all figures be, however precise the laboratory knowledge on which they are based, if they endeavour to cater for groups or communities. Even in such a simple matter as calorific requirements, all that can be said is that the total daily requirement of calories for a community containing  $N$  people is  $M$ , so that the *average* daily requirement for the individual is  $M/N$ . In fact, the requirements of the population as a whole will presumably be distributed about  $M/N$ , in accordance with the usual normal frequency distribution, though little is known about the breadth or steepness of the curve that indicates the normal variations in need for this or for any other dietary essential. But it is clear that up to half the population must require something less than  $M/N$  calories per day, while from a third to a half require more. There will certainly be no harm, from the social point of view, even though it may be wasteful in times of war or economic stringency, if the former part of the population, through receiving  $M/N$  calories per day, receive more than their needs; on the other hand, serious harm may arise if others, also receiving  $M/N$  calories per day, receive less than their needs. Indeed, those members at the upper end of the frequency distribution curve may come perilously near to starvation; how near we cannot say. Until, therefore, there is complete freedom of choice both in quality and quantity of food-stuffs for all members of the community, when the total requirements for any one food constituent will automatically be the average requirements multiplied by the population, it is necessary to cater in such a way as to make quite sure that those persons who have the misfortune to be on the upper side of the mode shall receive optimal nourishment, even though this means that we shall be offering to those below the mode more than they require or will take. It is surely better, to paraphrase what is alleged to be the basic principle of British justice, that a dozen men shall be over-fed than that one shall starve to death.



## CHAPTER XIX

### THE ABSENCE OF THE OPTIMAL

§ 8 THE results of animal experiment and of medical and social investigations have made it clear that inadequate diets most need improving in certain very simple directions. They are deficient in protein—especially in “good” protein—and in some minerals, notably iron and calcium; they lack sufficient of vitamins A and B<sub>1</sub>; they are probably low in vitamin D (especially for infants and young children) and possibly in vitamin C, riboflavine and other parts of the “vitamin-B<sub>2</sub> complex.”

During the last few years many expert bodies have described “minimum,” “adequate,” “safe,” and even “optimum” diets. Among these bodies are the Ministry of Health and the British Medical Association in this country, one having an even wider authority—the Health Organization of the League of Nations—and, the most recent and in my opinion the most important of all, the National Research Council of the U.S.A.: its Food and Nutrition Board have laid down in “Recommended Dietary Allowances” something approaching optimum standards for all the main essential dietary constituents. These reports have been published and can be profitably read by those with little or no technical knowledge. Indeed, their recommendations are likely to be incompletely translated into practice until responsible public opinion understands and supports them; the assent of scientists alone can be of little immediate effect.

§ 9 In the last chapter an attempt was made to lay down certain features of an optimal diet—with the qualification that this can only be done provisionally, in the light of existing knowledge. It is likely that further advances in knowledge may point the way to still further improvements. This possibility is clearly foreshadowed by experiments carried out in one of the most

famous laboratories of the United States; some striking and wholly unexpected results obtained there during the last few years have hardly received sufficient notice from students of dietetics.

A colony of rats had been kept going for several years—that is, for many generations—on a diet that seemed “optimal,” judged by the usual criteria. The animals were healthy and lively; they produced many and large litters of viable young; they lived to what was considered for rats a ripe old age. It was decided to find out what effects, if any, could be produced by certain simple supplements to this diet, which seemed already to allow the development of physically perfect rats. Green leaves and liver were the main additions; the diet itself contained abundant milk.

When these additions were made, the health of the colony was improved in almost every respect. The animals grew bigger and lived longer; more young were born; the incidence of infections was significantly reduced. The apparently perfect diet had been made more perfect! Further investigations suggest that the extra calcium and the extra riboflavine afforded by the supplements should receive credit for a good part of the improvement.

§10 With the proviso, then, that conceptions of the optimum may change as it is approached, it may safely be stated that the diets of most people fall short of this level, even as it can at present be prescribed. There exists to-day the available technique for analysing any diet, not only for its contents of fat, protein and carbohydrate—a very simple matter for the chemist—but also for the essential minerals and vitamins. The chemist, again, can give precise figures for calcium and iron, and these figures, along with the values for the three major constituents, are to be found tabulated in many books on “Food Values.” As a result of recent work, moreover, due allowance can be made for certain losses—trimming before cooking, wastage during cooking and on the plate and any difficulty in absorbing and digesting particular portions of the diet. All these facts are known

with sufficient accuracy to make possible the comparison of any diet with the current conception of the optimum.

This can also be done for vitamin contents, with sufficient exactness for practical purposes. For vitamins C and K, for aneurine (vitamin B<sub>1</sub>), for riboflavine, for nicotinic acid and for vitamin A and carotene, there are chemical or physico-chemical tests that permit the expression in quantitative terms of their amounts in any foodstuffs suitably analysed, without recourse to the far more elaborate and costly methods of animal assay, though this is still required for the measurement of vitamin D. Moreover, a new technique, known as "microbiological assay," has been developed in the U.S.A. and is being increasingly used here: it depends on the fact that some bacteria require certain vitamins if they are to multiply and survive. The principles of the methods used are exactly similar to those described in Chapter XV, but the method, though requiring great care and expertness, is enormously quicker and cheaper than are biological tests on mammals or birds. By means of these microbiological tests we have already had made available data about the distribution in foods of some of the "newer" vitamins—pyridoxin, pantothenic acid and biotin—as well as extended information about aneurine, riboflavine and nicotinic acid. Broadly speaking, we can now evaluate any given diet as a source of vitamins, in comparison with our accepted optimum. The vitamin values are not published in anything like so accessible a form as other food values, but they can and will be determined whenever the necessity for it is fully appreciated. There is sufficient agreement, too, about the daily optimal requirements for most of the vitamins to give practical utility to a knowledge of their amounts in various foods.

§11 We do not, in short, lack knowledge to improve human health by nutritional means. Nor do we need to indulge in fantastic habits of eating to obtain the best results from an optimum dietary. The order of taking foods at a meal is hallowed by custom that has, for the most part, a sound physiological basis. In this connection I am grateful for permission to quote from

*The Lancet* of 26th June, 1937, its editorial views on certain curious proposals for making a drastic alteration in our ordinary dietary conventions.

“ Few doctors in this country can have escaped being asked at some time or another what they think of the Hay diet. The questioner is more often an acquaintance than a patient, and the question casual rather than serious; for the person who intends to follow directions given in his newspaper does not want to hear his regular medical adviser say that these are unreasonable. Dr. W. H. Hay and his business associates have shown, if nothing else, that there are a great many people who can readily be persuaded to follow such directions; with suitable assistance one simple idea can be made to go a very long way. Dr. Hay's idea—or, as some call it, bugaboo—is that carbohydrates and proteins should not be eaten at the same meal, because protein (he says) increases the gastric secretion of acid and so hinders the digestion of starch by the saliva. The delayed digestion of carbohydrate is supposed to allow fermentation to take place, and the acids (of secretion and fermentation) are absorbed into the circulation, causing almost every known ailment, and eventually death. This month Dr. Eugene Foldes of New York has demonstrated how completely experience and experiment contradict this theory. The amount of hydrochloric acid secreted after taking carbohydrates is not very different from the amount secreted after taking proteins. Even if there were a significant difference, an increased amount of hydrochloric acid in the stomach would not wholly interrupt starch digestion; and even if it did so, fermentation in the stomach would not follow. If there were any fermentation it would be in the intestines rather than the stomach, and it would not lead to the production of significant quantities of acid; nor is there any reason to believe that the body would be unable to cope with such

acids even if they formed in larger quantities. So far, then, there is no evidence to substantiate the theory. Against it are the intimate mixture of protein and carbohydrate in many natural foods, the fact that contents of the stomach ferment only where there is achlorhydria, and the comparative harmlessness of 'acids' in a ketogenic diet except in one or two well-defined conditions, like diabetic coma. The chief danger run by followers of the Hay diet is that they may reduce the protein in their daily ration unsuitably!"

I can hardly imagine a more thoroughly effective exposure of the attempt to make Hay diets while the moon shines.\*

§ 12 Many "dieting" schemes tend merely to obscure the real nature of dietary deficiencies, though they occasionally bring incidental advantages. A "slimming" diet, for example, that reduces the intake of fat and carbohydrate—that is, generally, of chocolates and bread—may result in a higher consumption of foods containing more protein—if the consumer has the money to pay for such foods. And this may improve not only the balance of protein to fat and carbohydrate, but also the actual intake of minerals and vitamins that, we have seen, tend to fall to a low level in so many diets.

§ 13 It is known to-day—and has indeed been known empirically for a long time—that an abundance of dairy and garden produce is essential to a good dietary. But only in recent years have we learnt why. These foods—called "protective" for want of a better term—are rich sources of essential minerals and vitamins: the staple carbohydrate foods, cereals and sugar, of our vast industrial populations are often very poor sources of minerals and practically devoid of several vitamins. The poorer man's diet is doubly deficient; he cannot afford

\* Experimentally it has, moreover, been shown that meals of carbohydrate and fat, planned to contain minimal quantities of protein, cause considerable losses of nitrogen, presumably derived from tissue protein, that is, from muscle—surely a curious method of "slimming"! Most banting aims at reducing fat rather than muscle.

the protective foods, which contain these essential "minor" constituents, and the foods that satisfy his need for calories—expressed physiologically as simple hunger—supply him with little or none of those constituents. At the same time, these foods are short of protein, particularly of first-class protein. Man cannot live on fat and carbohydrate alone, but innumerable men are compelled to exist on diets that contain little else. If the reader thinks that this statement tends to exaggeration, let him recall for a moment peoples outside the British Isles, but not necessarily outside the British Empire.

Whatever may be the defects in the cooking and eating of most diets, these are not the most important faults. For the vast majority of the world's workers what is wanted is not mainly an altered order of courses, or even a better cook and kitchen, but simply more money. With that will inevitably come the purchase of more and better food by those who need it, and the rest will follow.

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